BARD Final Report US-4791-15R

Development of a universal volatile compound detection technology for early recognition of pests and diseases in fruit trees

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Project award year: 2015
One year feasibility project

Abstract

The original objectives of the 3-year proposed project submitted were:
1. Identify volatile organic compound profile(s) that distinctly correlates with the early infection of the red palm weevil.
2. Demonstrate that the VOC can be detected in situ by the GC/DMS instrument using the air-jet sampling technique.

With the funding of $100,000 for a period of 1 year. In order to achieve these objectives, we have restructured our original Aims as follows:
Aim 1. Detecting the key biomarkers of tree reaction to penetration and activity of harmful insects, where the model insect and tree are RPW and canary and date palm trees using GC/MS.
The in-field sampling and GC/MS analysis will be conducted by the Israeli team, and the chemical interpretation of the data will be conducted by the US team.
Aim 2. Demonstrate that the VOC can be detected in situ by the GC/DMS instrument and GCMS using the air-jet active sampling technique. The in-field testing will be conducted in Israel on RPW and date palm trees using GC/MS and in the US on citrus trees using GC/DMS. This will involve a selection procedure to optimize the air jets and the atmosphere around the sampling area, including tests with pulsed/focused jets, chemicals in its gas phases, nitrogen jets, hot and cold jets, to improve signal-to-noise ratios and elucidation of changes in VOCs while avoiding damage to plant and corresponding changes in VOC production. The GC/DMS device will be assembled by the US team specifically for the detection of volatiles documented under Aim 1.
Aims to be started in the first year and completed by the end of the second year:
Aim 3. Assembly of a GC/DMS device and laboratory-based testing of RPW - specific biomarkers to assess feasibility of field detection using portable DMS technology.
The UC Davis team will build a dedicated GC/DMS device and will benchmark it with the biomarker chemical mix that ARO would suggest under Aim 1.
Aim 4. Conducting lab and field tests and adopting the newly developed GC/DMS and the related analysis procedures to improve accuracy and sampling speed of RPW in date palms.
This Aim remains unchanged.
Aim 5. Linking findings from the thermal imaging via precision agriculture technology using IT algorithms for a gross monitoring of RPW activity, to reduce the number of trees to be handled by the GC/DMS to a level that facilitates commercial implementation.
Originally, the activities on this aim were planned to be carried out in the Year 3 of the project. Since the time frame is adjusted to only 2 years, we abandoned this Aim.

Summary Sheet

Publication Summary

PubType	IS only	Joint	US only
Thesis - MSc.	0	0	1

Training Summary

Trainee Type	Last Name	First Name	Institution	Country
M.Sc. Student	Spitulski	Sierra	UC Davis	USA
Postdoctoral Fellow	Pasamontes	Alberto	UC Davis	USA
Ph.D. Student	Peirano	Daniel	UC Davis	USA
M.Sc. Student	Anishchenko	Ilya	UC Davis	USA
Postdoctoral Fellow	Konishi	Mei	UC Davis	USA
Postdoctoral Fellow	Rajapakse	Maneeshin	UC Davis	USA
M.Sc. Student	Tabib	Adar	Agricultural Research Organization	Israel

Details of Cooperation:

The GC/MS data collected from trees in the enclosure conducted by the Israeli team have been then communicated to the US team. Results from the work by the US team was communicated to the Israeli team as well. Multiple technical exchanges and skype calls were done during the course of the year to coordinate the work.

Implications, both Scientific and Agricultural.

Due to the critical need for early detection of RPW in date groves in the US, Israel and many other countries, the present study lays a foundation to tackle this issue. Also, other commodity crop programs may tangentially benefit from the technical developments of this RPW work. Specifically, HLB has a large economic impact in the US, and citrus greening has the potential to inflict massive damage in Israel if/when it is introduced. Our team's work has been targeting developing a pre-commercial platform for HLB detection using citrus VOCs, using a first generation commercial platform with a non-radioactive source that is currently under development by a commercial company (Applied Nanotech Inc., Austin, TX USA). These platforms will make use of standard air sampling approaches, and improvements in these areas may have a dramatic impact on system performance and speed of monitoring. Since the sampling of HLB-infected trees in commercial groves has not been optimized, the present study is also filling the gaps in the infrastructure for plant disease detection via VOCs. The active sampling and the IT algorithms have been tested and implemented for the enhancement of detection of the HLB in citrus (and then RPW in palms), and this research is expected to lead to additional economic benefits for both the US and Israel.

Agricultural and/or Economic Impacts of the Research Findings, if Known.

The developed and tested hardware and VOC sampling system for citrus sampling and, later for RPW detection has been further tested on citrus trees. It is expected that application of the software, sampling approaches and, later, designated sampling manifold will greatly increase the efficacy of the sample intake and, therefore, will improve classification accuracy of HLB infection status based on VOC "fingerprints" profiling compared to our earlier HLB data and allow for detection of RPW. The data analysis models developed for the citrus model will be then applied to the VOC profiles data obtained with the enhanced sampling methodology from palm trees infested with RPW.

Achievements

Significance of Main Scientific Achievements or Innovations.

We have conducted regular volatile collection from five young date palms. In addition we also collected volatiles from two date palms and two Washingtonian sp. previously infected with RPW larvae, as the other experimental palms but were grown in greenhouse for about 30d till volatile collection. Overall the amount of volatiles, produced by date palms over 24 hours collection, are quite small (about 1µg total per collection on average). On average 100 peaks per chromatogram was observed. The fact that the peaks are spread all along the chromatogram indicates that the volatile profile is very heterogeneous in term of volatility. Not much difference was observed between volatiles collected from upper and lower part of the chamber. One year of study enabled us to

develop a system for head space collection from a growing date palm and identify two volatiles that potentially indicate RPW infection in palms. However more study is needed in order to proceed with volatile identification and further confirm identification of volatiles tentatively identified in this study.

The main thrust of the activities under Aim 2 was to explore, quantify, and enhance the chemical detection limits of a nonradioactive gas chromatography/differential mobility spectrometer (GC/DMS) by using hot air sampling from foliage of citrus trees. This was accomplished by: performing chemical benchmark testing of a GC/DMS prototype, coding a data analysis algorithm to quantify results from the device, performing in-field sampling of citrus trees in the field, and a proof-of-concept test of a proposed novel airflow system.

The GC/DMS was assessed using headspace sampling with a variety of agriculturally-relevant chemicals at varying concentrations to refine the operating protocol and determine the detection limits and reproducibility of the device. A method for consistent GC/DMS data quantification through a signal-to-noise filter and a cluster-seeking loop for feature identification has been developed and utilized. In addition, a suite of chemical tests using specific agriculturally-relevant chemicals and headspace sampling were performed to characterize device performance. The algorithm provided a quantitative rubric to assess noise levels, number of peaks, intensity of peaks, and variance among sample spectra. Following device characterization, sampling was attempted with a complex biological specimen. Results indicated that wounding responses were measurable, so a less-aggressive method of volatile collection was suggested that used additional airflow to propel stagnant surface volatiles toward the volatile detection device, the GC/DMS. For variable control, this method was tested in a lab setting with agriculturally relevant chemicals as a proof-of-concept. Upon testing success, designs were created for applying this method in-field with a range of user-varied parameters, such as funnel size, flow velocity, and heat. A design for a device that uses heated airflow to optimize collection of volatiles is proposed and preliminary proof-of-concept testing confirmed signal enhancement. This work builds the foundation for further integration of the air jet manifold under development by the Israeli team. Future engineering design considerations are recommended for a theoretical in field device. The achieved results further established the infrastructure for field testing.

During this one year study, we developed a system for head space collection from a growing date palm and identify two volatiles that potentially indicate RPW infection in palms. However more study is needed in order to proceed with volatile identification and further confirm identification of volatiles tentatively identified in this study.

Publications for Project US-4791-15R

Status	Type	Authors	Title	Journal	Vol:pg Year	Coun
Published	Thesis - MSc.	*Spitulski, Sierra*	Quantification, characterization, and enhancement of volatiledetection capabilities fora non-radioactivegas chromatography differential mobility spectrometry (GC/DMS)chemical analysis systemfor agriculture applications		:	US only

Background to the Topic

Volatile Organic Compounds

As an end product or byproduct of metabolic processes, all living beings emit chemical compounds in some form. These emissions commonly include volatile organic compounds (VOCs), which are carbon-containing chemicals that have sufficient volatility at standard temperatures and pressures. The study of VOCs is important due to applications in measurements of air quality, early detection of disease biomarkers, presence of explosives, health monitoring of wildlife, and even plant responses to both internal and external stimuli. While VOCs can be anthropogenic, this report will focus on the biologically generated volatiles, particularly those from plants.

Volatiles emitted from plants can come from a variety of botanical tissues, leaves, fruit, flowers, roots, and commensal microorganisms and are possibly indicative of internal physiological changes, defensive responses, and communications with other plants (Sumner; Baldwin; Kessler). For example, when a plant undergoes abiotic or biotic stress, it releases certain defensive compounds known as induced VOCs; some plants in close proximity have been documented emitting warning volatiles to surrounding plants to encourage premeditated defenses (Baldwin, Halitschke).

These volatiles are crucial to plant existence and can provide great insight into normal metabolic processes such as flowering, maturation and ripening, as well as levels of response to pests, disease, environmental stress, and nutritional deficiencies. In essence, measurement of these VOCs provides a non-invasive method to monitor all stages of plant life—including through crisis such as infection.

The study of plant responses is important to the increase of crop output and prevention of damages caused by disease and insects. Presently, infectious pathogens such as the citrus greening disease (HLB) and Citrus Tristeza Virus (CTV) in citrus trees, plum pox (sharka) in plum trees, and grape leafroll virus in grapevines, as well as pest infestations such as Red Palm weevil (RPW) in date palms and the Asian citrus psyllid (ACP) in rutaceous plants have caused great ecologic and economic strife for growers in the agricultural industry. Some of these infections and infestations can run rampant through orchards, necessitating massive plant removal or extreme pesticide application. Studying the volatiles emitted from the plants at varying stages of disease would allow for disease biomarker identification and enable early intervention (Aksenov; Cheung; Pasamontes).

Currently, both invasive and noninvasive VOC collection techniques exist. Invasive—also known as destructive—VOC sampling involves detaching plant tissues for analysis (Tholl; Boland). This can be performed on fresh or dry samples (Zini; Augusto). While this is an effective technique for monitoring stress response, it is not the ideal method for observing natural life cycles, as the act of detaching tissue induces a wounding response (Schilmiller). Noninvasive monitoring techniques include using whole plants, fruits, or leaves for *in situ* collection, leaving the subject plant unharmed.

In both invasive and noninvasive sampling, VOCs are collected on sorbents—chemical materials such as polydimethylsiloxame (PDMS) used to cause chemical analytes to either absorb through a surface or adsorb to a surface. Examples of sorbent mediums include solid-phase microextraction (SPME) fibers, stir bars for sorptive extraction (SBSE), microporous activated charcoal, and Carboxens. The VOCs can be collected passively through positioning of sorbent mediums within the plant canopy for sampling of diffused emissions, or actively by using vacuum systems to pull a volume of volatiles across the sorbent surface (Harper). For this report, we will be focusing on the latter.

Problem

Infected plants usually present a robust host response to infection by pests or diseases before they present severe symptoms and/or collapse. Such reaction cause changes in the plant metabolism and chemical characteristics but in certain cases it is detectable at a very late stage by commonly used technologies. Existing diseases and pests such as Huanglongbing (HLB) or greening in citrus, Red palm weevil (RPW) in dates, Sharka in plums, Leaf roll in grapes, Citrus Tristeza Virus and many more, cause enormous damages to growers and agriculture mainly because they are undetectable by the existing technologies until a very late stage. The long incubating period leaves the growers with only costly treatment alternatives, i.e. massive application of pesticide and removal of trees.

The red palm weevil (RPW), *Rhynchophorus ferrugineus* Olivier (Coleoptera, Curculionidae), is an economically important pest of various palm species (Arecaceae) that has invaded many continents and is present in California, the Caribbean islands and Israel. Southern Florida is also highly suitable for infestation of this pest (Fiaboe, 2010). In 2010 it was officially confirmed that the RPW caused death to palm trees in California (Ferry, 2010). Male red palm weevils produce an aggregation pheromone, which attracts other adult weevils to their host; it is composed of ferrugineol (4-methyl-5-nonanol) and 4-methyl-5-nonanone (Murphy and Briscoe 1999). Infested palms emit a fermented odor particularly around tunnel openings and other damaged areas (Abraham et al. 2000). Dogs can be trained to detect the odor of red palm weevils in palms (Nakash et al. 2000; Suma et al., 2013), but the training must be reinforced very frequently. The option of using the suggested device and technology instead of dogs was stressed by Vacas, et al. (2014) who showed that the changes in volatile profiles of the palm material affected by the RPW have been attributed to esters, acids, alcohols, and ketones, as opposed to predominate terpene hydrocarbons by the healthy plant tissue.

The invasive RPW appeared in the Middle East in the 1980s and since has caused severe damage to date production causing a substantial yield drop (Murphy and Briscoe, 1999; EPPO, 2008) and extensive damage to Canary palms. In Israel, the history of the weevil invasion dates back to 1999 (Soroker *et al.*, 2005). Following the occurrence of RPW and its spread in neighbouring countries, surveillance traps were used in Israel since spring 1999, baited with RPW aggregation pheromone and a plant kairomone mixture (Soroker *et al.*, 2005). Subsequently, a line of 100 traps was set along the Jordanian border and in adjacent date plantations. More weevils were trapped and infested trees were identified, first north-east of the Dead Sea shore and later in the Jordan Valley.

Due to the known difficulties in controlling RPW, an intensive Integrated Pest Management (IPM) program was initiated where three governmental agricultural agencies joined forces: Israeli Plant Protection and Inspection Services (PPIS), Agriculture Extension Services, and Agricultural Research Organization (ARO). Peres Center for Peace was also involved in facilitating additional collaboration with neighbouring countries. Massive monitoring and trapping efforts were launched, and about 5,000 pheromone traps were set and GIS mapped. Experts examined trees for infestation symptoms, such as drying offshoots, drying crowns or oozing from the trunk. With assistance from the traps, this effort detected 35 infested date palms in 1999. The palms were colonized only at the lower part of the tree stem. Following chemical treatment about 60% were cured while the rest were uprooted and burned.

A prophylactic treatment was used over 1130 hectare up through mid-2001, where palms received three soil applications of Confidor 350 SC (Imidacloprid, Lidor Chemicals Ltd) while offshoots and palm trunks were sprayed up to two meters in height, once a month, with either Cutanion EC (Azinphos methyl, Makhteshim-Agan Industries Ltd, Israel) or Dizictol EC (Diazinon, Makhteshim-Agan Industries Ltd, Israel) or Dorsan EC (Chlorpyrifos, Luxenburg Ltd, Israel), as described by Soroker *et al.* (2005). Moreover, in order to prevent RPW infestation following offshoot

removal, the cutting wounds were treated with Dorsan EC as above. Between 2002 and 2009, no infested palms were detected. The situation has changed with the report of a dead Canary palm in a private house in the north-west of the country in 2009. Since then **hundreds of palms have died** and the infestation continues to spread. The infested palms and their offshoots are mostly treated as recommended - drench of Confidor and trunk spraying of Karate max (Soroker et al, 2013).

Early detection of Red palm weevil (RPW, *Rhynchophorus ferrugineus* Olivier, 1790) infestation is particularly crucial in palms at early infestation stages, when the apical meristem (the palm heart) is not yet damaged and the trunk is still stable. Under these conditions the trees can be treated and usually recover. However, as the RPW develops inside the palm, well hidden from the human eye, the early detection process with the current technology is a challenge, if not impossible. Detection is especially problematic in cases of date palms that show no clear visual symptoms, often until they crash.

Prevention of further spread of this invasive pest requires RPW monitoring especially at ports of entry and at new infestation foci. Various methods and approaches have been evaluated over the years for early detection of RPW infestations: visual, acoustic (Gutiérrez et al., 2010; Hetzroni et al., 2004; Hussein et al 2010; Mankin et al., 2008;2011; Pinchas et al. 2008; Potamitis et al., 2009; Siriwardena et al., 2008; Soroker et al., 2004), volatiles detection by trained dogs (Nakashe et al., 2002; Suma et al., 2013) and chemical and volatile changes found in lab (Vacas et al. 2014). However, despite the intensive effort devoted to development of RPW detection technologies, all are still falling short from being practical or feasible commercially, leaving detection to be dependent mainly on visual inspection in most areas. Details on the setbacks involved in implementing the above potential technologies and additional ones are provided in the following section.

Crashing of tall ornamental palm trees poses serious safety hazards. In addition, Israel and US date growers produce high quality products valued at more than $5,000 per ton on the European wholesale market. Israel's production is also monotonically rising, and the country produces now more than 30 thousand metric tons per year and that of the US is even higher (Botes and Zaid, 2007; Glazner et al. 2006; Glazner 2012). Israel and Europe have invested substantial R&D funds for early detection of the RPW, but as described in the following section, no technology proved to be a feasible solution to the growers in high acreage commercial production conditions.

Due to the critical need for early detection of RPW in date groves in the US, Israel and many other countries, this insect and crop were selected for the present study. The major focus is entirely on this effort. However, other commodity crop programs may tangentially benefit from the technical developments of this RPW work. Specifically, HLB has a large economic impact in the US, and citrus greening has the potential to inflict massive damage in Israel if/when it is introduced. The effort of the US part of the team, therefore, was focused on measuring volatiles from citrus

Differential Mobility Spectrometry

The employed detection technology is based on the differential mobility spectrometry (DMS). With ions in DMS, the forward x-motion is imparted by the carrier gas, while the y-motion is caused by the alternating electric fields. Because of the motion in the y-direction, the alternating high and low electric fields from the parallel plates would, under specific circumstances, fail to drive the ion to the detector. Those circumstances are when the difference between the ion's mobility in the high electric field (K_h) and the low electric field (K_l) does not equal zero. Considering that an ion can only be detected when the difference in ion mobilities is zero, a range of electric fields must be swept in order to measure different ions. To ensure different ions are "sensed" (the charged ion induces a current onto the detector plates relative to its abundance) by the detector, a DC voltage or "compensation voltage" (CV) is applied. This CV can be applied at various voltages in a sweep to

separate and aide in the detection of the ions, directly affecting the ion based on structure and mass. Because of this specificity, the ions are then attributed to that range of CV during which they are detected.

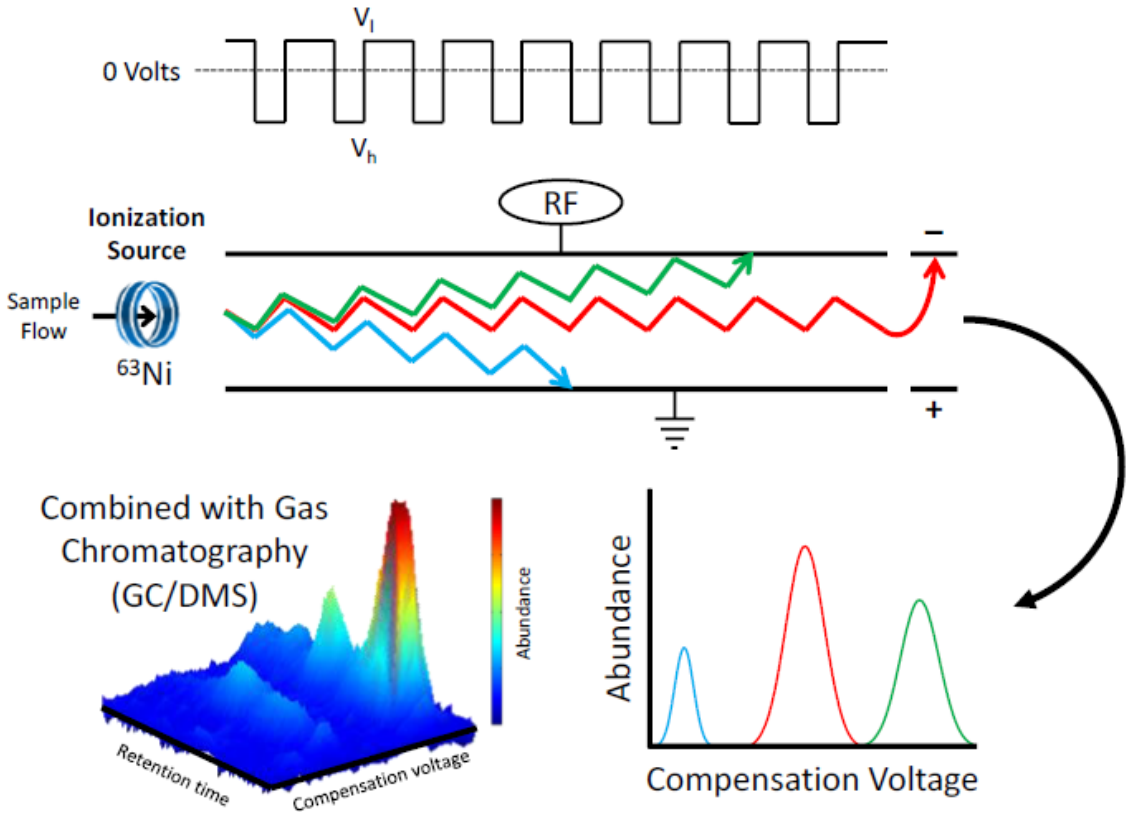


Figure 1: DMS summary (Peirano 2013)

When GC is coupled with DMS, the results are multi-dimensional. The DMS dimension of detection (CV) in conjunction with the retention time (RT) component from gas chromatography allows for two modes of separation. Because the two dimensions are mostly orthogonal (Anderson), detection of ion abundance (translating later into concentration) can be visualized. This technology and ion behavior is schematically demonstrated in Figure 1.

Coupling GC with DMS provides many benefits. Unlike the bulky mass spectrometer, differential mobility spectrometry can be quite compact and portable. When paired with miniature GC systems, the coupled technology can be fit into a suitcase and easily taken into the field for *in situ* testing, as shown in Figure 2.

Figure 2: Field-deployed portable GC/DMS sampling from citrus tree

Portability is a much underappreciated trait for complex analytical devices. The compact GC/DMS system is field deployable, and highly suitable for noninvasive sampling of VOCs. Recent work has demonstrated success in using GC-DMS for *in situ* research in orchards for the detection of HLB in citrus (Aksenov), as well as in contained greenhouse research facilities that house plants infected with pathogens too dangerous to be removed for testing (McCartney).

In addition to portability, a GC/DMS device is typically expected to have very high sensitivity when it comes to some important biomarkers. The technology has been noted for achieving detection of extremely low concentrations in the range of parts-per-billion (ppb) or parts-per-trillion (ppt) (Barnett; Handy). It has also been able to deconvolute very similar analytes, such as xylene isomers in an experiment designed to confound the detection capabilities (Eiceman 2002). This technology also has the capability to differentiate among homologous compounds at very low concentrations (Eiceman 2001).

One of current persistent problems with the DMS technology is difficulties in standardization and lack or reproducibility. Slight differences in ionization type, GC column stationary phase, temperature profiles, and other parameters can drastically alter the capabilities of a device.

The Non-Radioactive GC-DMS

One of the most common sources of ionization for coupled GC/DMS devices is a Ni-63 foil that emits radiation in the form of β-particles that ionize carrier gas (in case of air nitrogen and oxygen molecules), which through a series of molecular reactions, predominantly proton transfer

lead to formation of ions from compounds with Lewis basicity. This method of ionization is preferred often because of its consistency with sensitivity and selectivity (Louis; Michels), as well as the predictability of the emitted radiation. Pre-assembled DMS devices using Ni-63, for example, were manufactured by Sionex Corporation (Bedford, MA).

However, radioactivity poses many challenges for researchers: disposal issues, corrosive byproducts, radiation safety necessities, heavy regulatory burden, etc. This is especially problematic in an international cooperation. After the Davis Lab had several successes using custom, radioactive GC/DMS systems for disease detection in citrus trees (Aksenov; McCartney), an alternative, nonradioactive GC/DMS was enlisted to be used in field testing of Red Palm Weevil infestation of date palms and citrus trees infected with the greening disease HLB.

This device was constructed and supplied by Applied Nanotech, Inc. (PEN, Inc, Austin, TX). The ionization source is a nonradioactive technology known as plasma discharge, in which an alternating voltage is induced across two parallel-plate electrodes. The current through the electrodes is controlled by the company's proprietary ionizer driver (Tikhonski), and creates plasma which ionizes any gas-phase matter passing between the electrodes (Michels; Tikhonski).

Aside from the nonradioactive ionization source, this device operated in the usual way of a portable GC/DMS. Figure 3 shows a schematic of the internal operations of the device. During sorption phase ("sampling"), the pump draws air through the inlet across the preconcentrator (the adsorbent "trap") to capture volatiles. When the sampling phase is complete, the detection phase is implemented: the preconcentration trap is rapidly heated to desorb the VOCs while the flow through the device is redirected to guide the collected chemicals into the heated GC column for separation then through the DMS for further separation and, finally, detection.

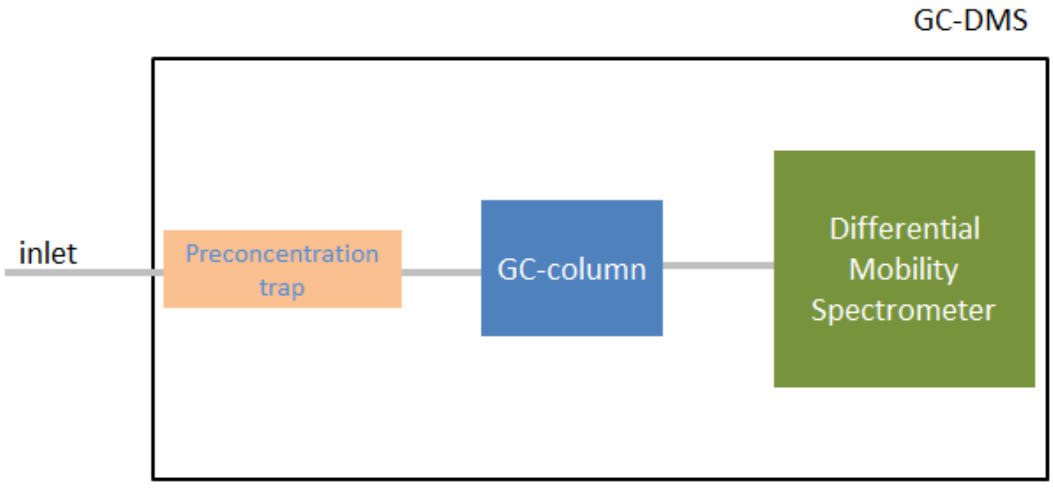


Figure 3: Simplified schematic for the internal operations of a GC/DMS

Major Conclusions, Solutions, Achievements.

The main focus of the US part of the team was on Aim 2. Multiple improvements and specific modifications needed to be implemented prior to enable in-field use of the non-radioactive GC/DMS device and to adopt it for RPW detection applications. Because this device is novel and previously uncharacterized, its chemical detection capabilities are not known. As a field-deployable device, the ability to perform routine plant samples with

reasonable sensitivity is imperative to future research. The objective of the conducted work under the Aim 2 involved answering the following three questions:

- What are the performance metrics of the non-radioactive source GC/DMS?
- What are the detection capabilities of this GC/DMS device for volatiles of a complex biological sample?
- Can the detection capabilities be enhanced by heated air flow directed into the canopy of a citrus tree? Future assembly of the field device using the air jet system supplied by the Israeli team is anticipated.

In order to address the first of those questions, a consistent rubric for quantification was required in order to eliminate the current subjective method of visual inspection. An algorithm for feature identification and quantification using signal-to-noise ratios was created for application to the data. Chemical tests were then also conducted to assess several key performance characteristics: sensitivity, selectivity, chemical noise, number of peaks, intensity of peaks, and reproducibility.

Following chemical testing, the device was field-tested with a complex biological sample—a citrus tree—under various stress conditions. After determining the field sampling capabilities, signal enhancement techniques were proposed and tested. For this, a mock airflow device simulating air jet sampler was used to provide proof-of-concept enhancement of volatile collection, and designs were created for a similar theoretical application for future work.

Metric for quantification

Data generated by the GC/DMS is in large dimensions (more than 100,000 singular data points) for a single sample. Creating an algorithm to consistently visualize and compare the features within hundreds of samples that inherently contained varying degrees of noise and drift was a crucial component to fully characterizing the device. Presently, quantitation comes in the form of visual inspection—an extremely subjective practice. A standard metric for objective evaluation of any and all GC/DMS data was thus created to fill this need. All codes were previously written and executed using MATLAB (The Mathworks Inc., Natick, MA, 2014) and adapted from open source code that our the Davis laboratory previously published (Peirano).

The Feature Selection Algorithm

With GC/DMS data, visualization techniques are one of the most effective means of understanding system output. Currently, existing software allows the user to visualize, preprocess, and statistically analyze trends within sections of or entire chromatograms (Peirano 2016). However, these tools do not enable selection and objective quantification

of individual features within the spectra. The proposed method of feature selection is not directed or intended as replacement for preprocessing or statistical analysis, but rather as a criterion for identifying significant features and removing aspects of the signal attributed to system-generated noise, in addition to removing dependency on subjective visual inspection for quantification. This criterion takes the form of a bounded signal-to-noise filter.

Signal-to-Noise Filter for Feature Identification

At present, many definitions exist for calculating the signal-to-noise ratio (SNR) of a system. The equation chosen for this application is most commonly used in image processing, for which GC/DMS data is a candidate. For the purposes of this report, the SNR was measured as a ratio of the mean peak intensity to the standard deviation of the noise. Because the objective of this algorithm was to determine significant features rather than identifying the SNR, rearrangement of this ratio yielded the proportionality shown in Equation 1.

$$SNR = \frac{\mu_{signal}}{\sigma_{noise}} \rightarrow \mu_{signal} = SNR * \sigma_{noise} \qquad (1)$$

This relationship requires a threshold value for acceptable SNR. Based on the Rose criterion commonly used in image processing, the SNR must be at least 3-5 in order to be considered a non-noise signal (Rose). For this reason, enabling the SNR to be a user-adjusted parameter (depending on noise filtration needs) with a value greater than 5 ensured adequate signal identification.

To obtain the standard deviation of the noise, four regions of the data were selected and averaged (see Figure 5). Due to the nature of GC/DMS and the established protocols for device run length and CV scan range, it is rare for significant chemicals to be detected in these regions. For that reason, these sections of data were the most reliable and consistent for establishing a representative noise level. In addition, noise amplitudes can widely differ between high and low CVs and retention times. For example, column bleed may appear at elevated temperatures/ higher retention times and contribute to the baseline noise. The four corners ensure a statistically relevant sample of the noise spectrum. The standard deviation of the noise samples were taken and determined to be the standard deviation of the noise, σ_{noise}.

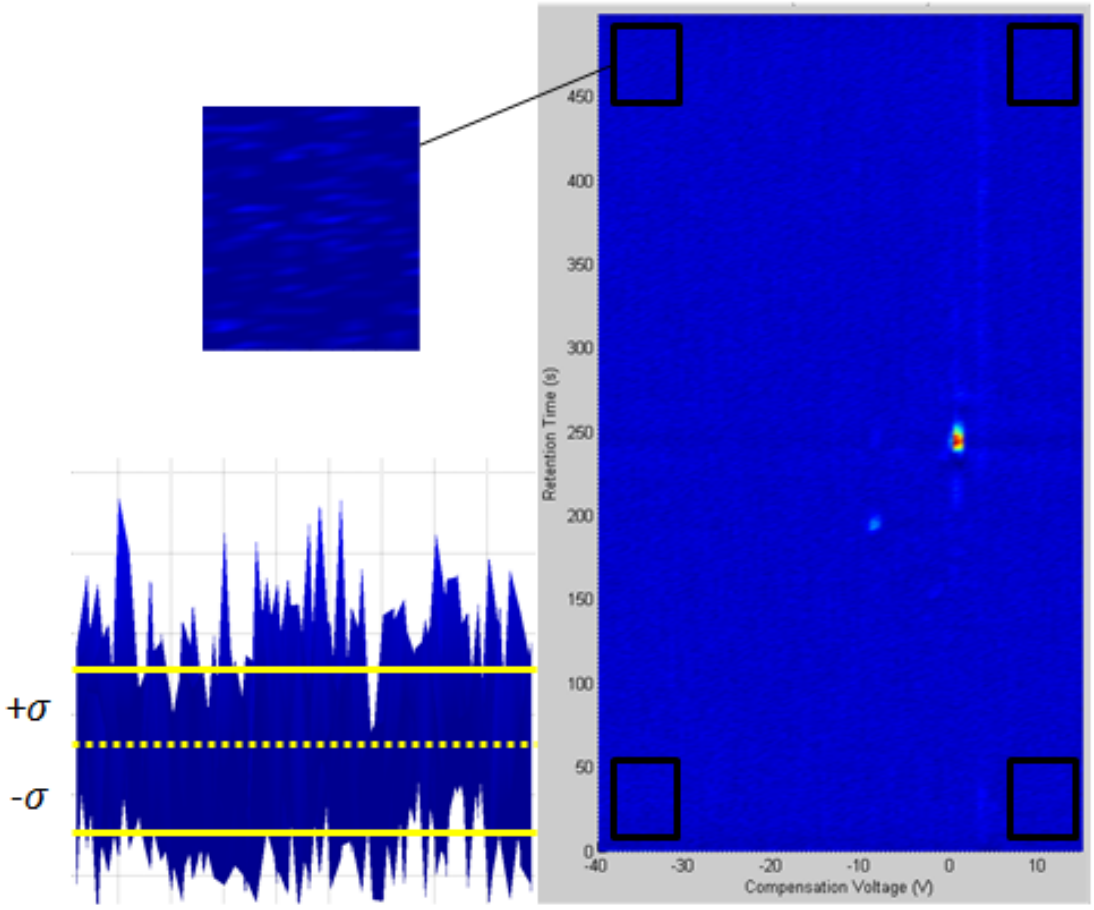


Figure 1: Regions selected as representative noise measurements

After calculating σ_{noise} and selecting a suitable value for SNR, the position within the spectra of any "pixel" (a single CV and RT coordinate reading) exceeding their product is preserved, while all other values are zeroed. This causes all data at or below the acceptable noise level to be removed, leaving only significant signals behind (see Figure 6). To account for any aspect of the signal attributed to the noise floor, the average noise signal is subtracted from the remaining, nonzero signal. The remaining intensity is used as the true feature signal.

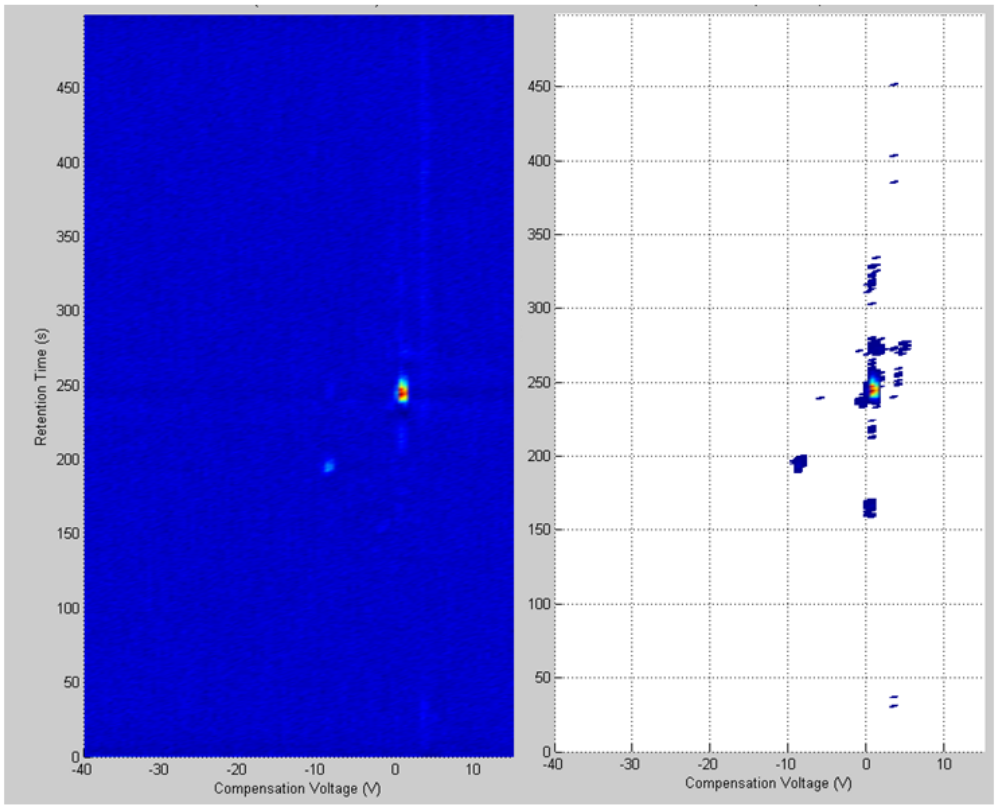


Figure 2: Baseline correction compared to removal of non-significant features

After the SNR filter is applied, the data are processed through a cluster-seeking loop that identifies any remaining nonzero pixel and evaluated surrounding pixels for value. This allows for clusters of nonzero data to be identified and counted, as well as allowing cluster metadata to be stored. For each chromatogram, the number of clusters (now confidently identified as features since making it through the SNR filter) is recorded. For each feature, the base area (number of pixels) and volume (sum of pixel values) are also recorded. One of the generated tables is sorted by features within the sample, and the other is sorted by highest feature volume. This information is compiled into the table shown in Table 1. Features are indexed by the location of the highest intensity pixel within the feature as a coordinate of RT and CV.

Table 1: Sorted feature table

'RT'	'CV'	'Peak density'	'Peak area'	'File name'
131	123	76	28.2636	'..\0222_PO...
162	119	54	19.4921	'..\0222_PO...
132	122	46	15.4912	'..\0239_PO...
162	119	37	13.0770	'..\0239_PO...
162	118	24	7.3600	'..\0240_PO...
131	122	18	4.9636	'..\0240_PO...
62	46	12	4.0187	'..\0237_PO...

An additional table can be compiled which equates peaks with identical (or within error) features based on RT and CV indices. This table most similarly resembles the “peak tables” generated by software for other high-level analytical devices, such as GC-MS. The GC/DMS peak summary representation is shown in Table 2. Empty cells indicated a specific feature not present in a given sample.

Table 2: Common features by sample

'RT'	'CV'	'..\0222_POS.XLS'	'..\0224_POS.XLS'	'..\0231_POS.XLS'	'..\0237_PO...	'..\0238_PO...	'..\0239_PO...	'..\0240_PO...
62	47	2.5829	[]	[]	[]	[]	3.2570	[]
69	46	0.4465	0.2574	[]	[]	[]	[]	[]
162	119	19.4921	[]	[]	[]	[]	13.0770	[]
131	117	0.2186	[]	[]	[]	[]	[]	[]
151	135	[]	0.2117	[]	[]	[]	[]	[]

Summary of Feature Selection Process

In addition to the feature tables, the final output of this process is a graphical summary of the compared samples, example shown in Figure 6, consisting of input data average (preprocessed or otherwise) and average location for detected features. The overall workflow for the constructed algorithm—from SNR filter to feature selection and quantification—can be seen in Figure 7.

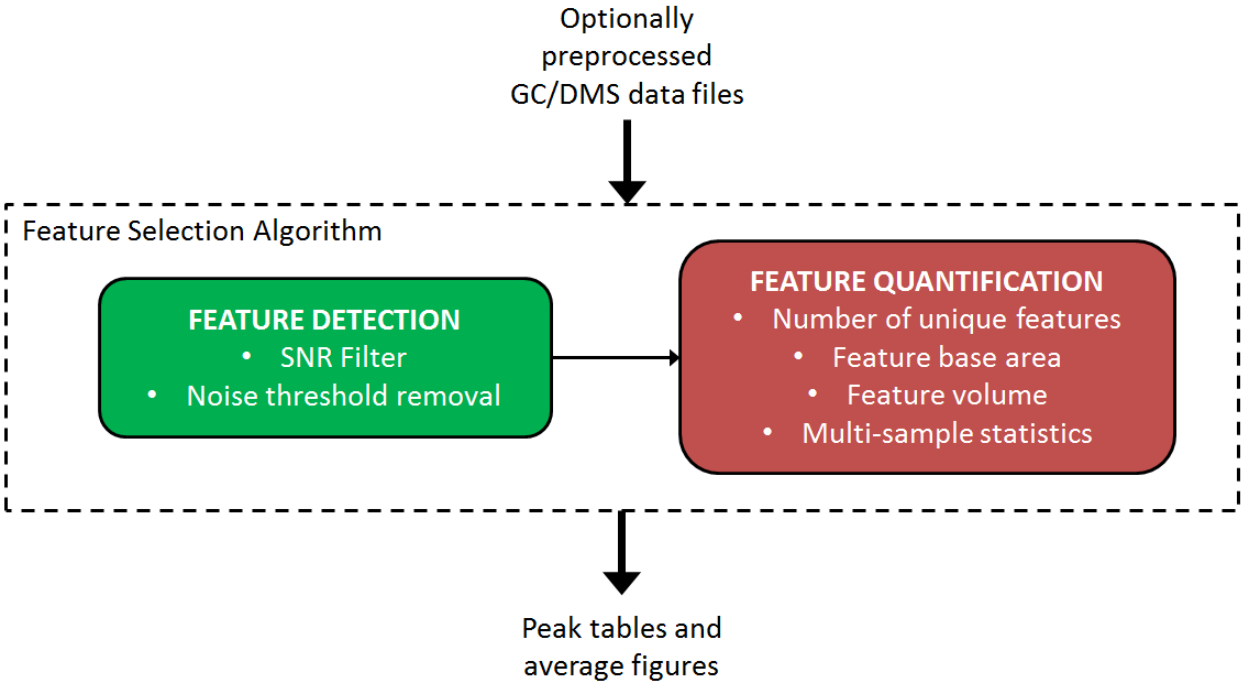


Figure 3: Workflow for quantification of features within GC/DMS data

While this feature selection process can be applied to any GC/DMS data, for this report it was applied to the range of chemical experiments carried out to fully characterize the capabilities of the subject device. The quantitative output of the feature selection process provided an objective measurement for evaluating the detection performance of the nonradioactive GC/DMS.

Performance Evaluations Through Chemical Testing

The goal of chemical testing was to determine the detection limits of the GC/DMS device quantitatively. This meant sampling a selection of chemicals to assess performance based on metrics such as reproducibility, selectivity, and sensitivity, as well as optimizing several parameters including sampling time and RF voltage. The chemicals chosen for this study were selected to represent typical compounds produced by plants, specifically citrus HLB disease biomarkers identified previously (Aksenov). The experiments were aimed at assessing three primary metrics: sensitivity, selectivity and reproducibility. All values were quantified using the feature selection algorithm.

Chemical Dilutions and Sampling Procedure

The chemicals that were selected for the device's chemical characterization process based on their presence in agricultural studies are shown in Table 1. The basis of the chemical testing was a method of headspace sampling. For this report, the process involved

aliquoting 3 mL of a liquid of known concentration into a 10 mL vial. A metal screw cap with a PTFE septum was screwed on for an airtight seal. Once the chemicals in the vials had reached to liquid-gas equilibrium (20-30 minutes depending on chemical volatility), the cap was perforated with a hollow needle tip to allow airflow, as well as the inlet tubing to the GC/DMS to draw the gas phase of the sample into the device. A schematic for this type of sampling can be seen in Figure 8.

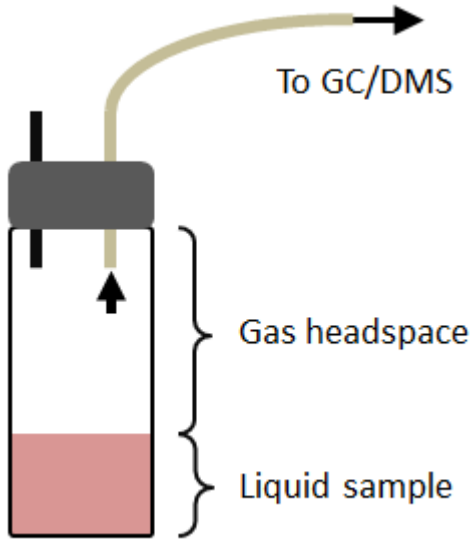


Figure 4: Headspace sampling schematic

Based on the liquid concentration and Henry's Law of partial pressures, shown in Equation 2, the concentration of the compound in gas phase was calculated. In this equation, p is the partial pressure of the headspace, k_H is the Henry's Law constant, and c is the concentration of the liquid sample.

$$p = k_H c \qquad (2)$$

This required knowledge of the Henry's Law constants for certain chemicals. These values are also shown in Table 3 and were obtained experimentally by others. It should be noted that the Henry's Law constants varied greatly among researchers and experiments. The values used were averaged values for aqueous solutions.

Table 3: Henry's law constants for tested chemicals; [1]Niinemets et al; [2]Bohon et al; [3]Buttery et al; [4]Riddick et al

Compound	Henry's Law Constant [atm mL/mol]
3-carene	134600[1]
m-Xylene	5882[2]
p-Xylene	6250[2]
octanal	526[3]
octanoic acid	0.89[4]
ethanol	5.0[1]

The concentrations were made working backwards from Equation 2. Dilutions from stock solutions into a methanol solvent were made using both simple and serial dilutions. A sample calculation is as follows: for 3-carene, a ~50 ppm headspace needed to be obtained. Using the k_H value from Table 1 and the molar mass of 3-carene, the liquid concentration needed to be ~50 ng/mL. The initial stock solution of 3-carene had a density of 0.857 g/mL. By extracting 0.1 mL of the stock solution and diluting it with methanol into a 10 mL vial, a liquid concentration of 8.57 mg/mL was made. This was further diluted using the same protocol until the target concentration of aqueous solution was obtained.

Device Use Methodology

The sorption and detection phase methodologies for the device were as follows. The sampling time (sorption phase) of the chemical experiments was 2 seconds. The detection phase began with the implementation of the temperature profiles shown in Figure 9. These temperature profiles were established empirically in prior sets of testing. The initial spike in temperature at 1 s for the trap was necessary to quickly desorb the sampled VOCs, while the decrease in temperature at the end allowed the trap to cool more quickly between runs. The GC profile was experimentally established for better chemical selectivity. Including the initial sampling phase and subsequent desorption and measurement, one complete sample collection lasted 500 s.

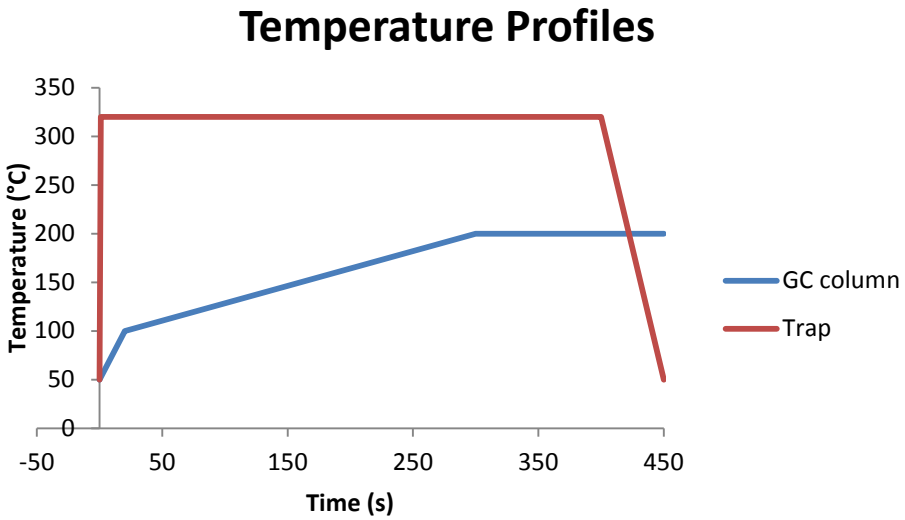


Figure 5: Temperature profiles for the GC column and adsorbent trap

The DMS sensor employed in the tested device had a 500 μm gap size, and operational parameters were set as follows: sensor temperature of 55 °C, carrier gas flow of 300 mL/min, asymmetric waveform amplitude (RF voltage) 1400V with the asymmetric waveform 34% high field and 66% low field, CV scan range from -37 to +15 V with a step of 0.28 V; 200 steps across the CV scan with 10 ms per complete CV scan for resolution; and a Carbopack B/Carbopack X mixed adsorbent matrix as a trap.

Experiments and Results

Headspace sampling was performed using the aforementioned method on the varied concentrations of chemicals. In order to test sensitivity (limit of detection range), 3-carene was sensed at 4 different concentrations until the lowest limit was established. From this, a calibration curve was made, shown in Figure 10. The sensitivity of this device for this experiment was approximately 10 ppm.

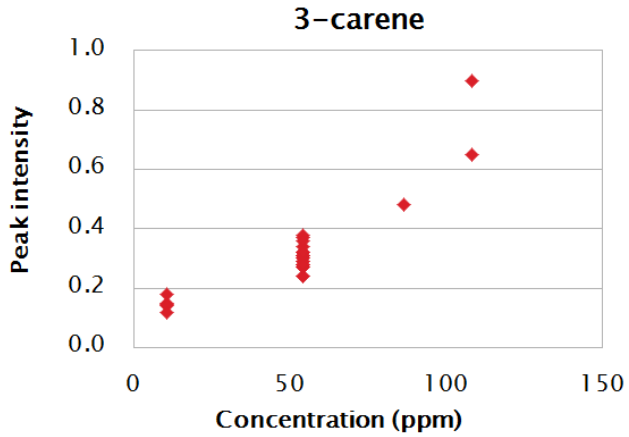


Figure 6: Calibration curve of 3-carene for varying concentrations to assess sensitivity

Additional experiments were performed on using 3-carene because of the clear resolution of the feature in the data, shown in Figure 11. A reproducibility measurement was made on a sequence of 20 replicates. Figure 12 shows the results of this.

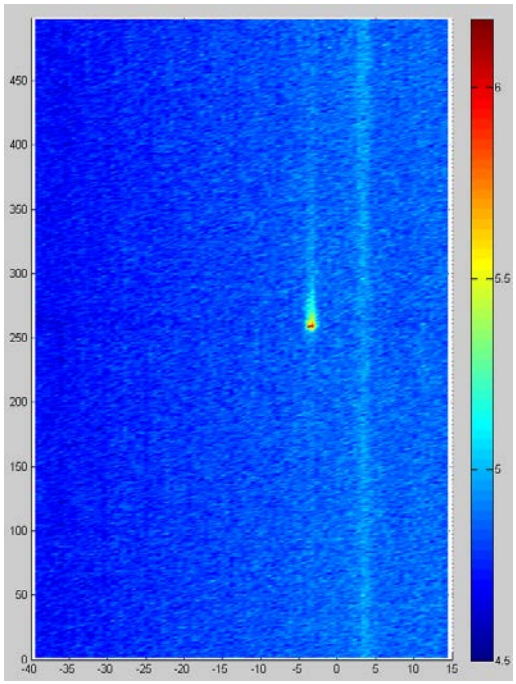


Figure 7: Unprocessed 3-carene sample

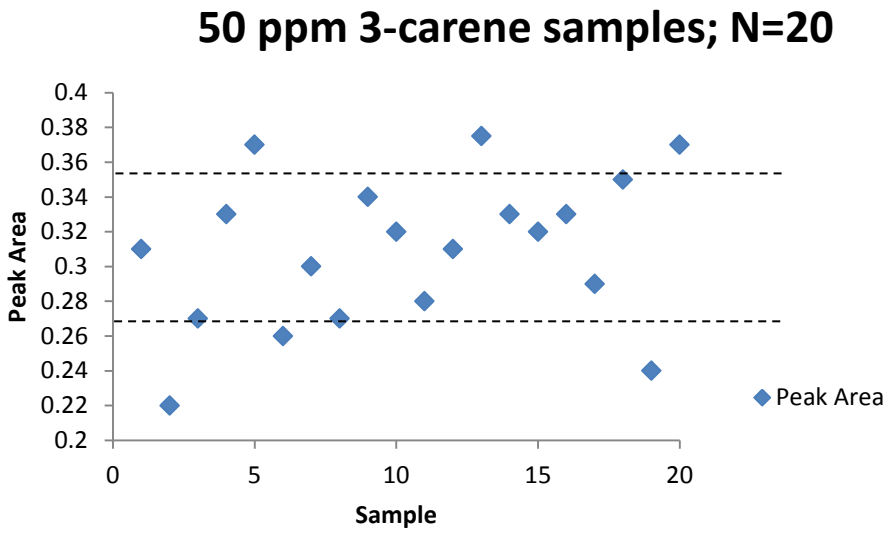


Figure 8: Deviation analysis of 3-carene with 20 replicates to assess reproducibility; dashed lines representative of a single standard deviation ($\bar{x} = 0.309$; $\sigma = 0.043$)

To test the selectivity of the device, two isomers were processed simultaneously. The isomers, p-Xylene and m-Xylene, were mixed in a concentration to 50 ppm headspace. The results were less than ideal. While both chemicals eluted at the same retention time, they appeared to form a single, indistinguishable feature. When tested individually, they appeared in slightly different (±1V) CV ranges, but when tested together, the features were unresolved. Still, the limit of detection for each was evaluated, shown in Table 4.

A few additional chemicals were tested to explore detection limits, shown in Table 4. Because the GC column on the nonradioactive GC/DMS was a DB-XLB model, information was available on the Kovats retention index (listed in Table 4). The experimentally determined and theoretical Kovats indices were found to be in agreement, which indicates instrument performance was as expected for the GC module. One notable exception to this was octanoic acid, which was not detected as expected. Octanoic acid, a relatively waxy compound, is retained strongly by the DB-XLB column, and is not eluting at the temperature profiles achieved by the GC heater or in the duration of the GC protocol. This indicates that octanoic acid or higher compounds may not be amenable for detection using the current device configuration (GC protocol and column type).

Table 4: Multiple chemical sensitivity assessment correlated with GC-MS retention index (*DB-5 column)

Compound	Retention Index (DB-XLB)	Retention Time (GC/DMS)	Experimental limit
ethanol	*669	~100	NA
m-Xylene	878.8	195	50 ppm
p-Xylene	879.7	195	50 ppm
octanal	*1006	250	50 ppm
3-carene	1011.4	265	10 ppm
octanoic acid	*1279	Not determined	Not determined

Most GC/DMS have variable RF voltages—the voltage applied in an asymmetric waveform to one parallel plate in the DMS drift tube. Historically, lower RF voltage is known to increase the signal of analytes. Higher RF voltage usually leads to higher ion loss and decreased ion transmission in the DMS device. This was assessed, alongside experimental detection limits.

Using 3-carene at ~50, identical sequences of experiments were conducted to optimize the performance of the device based on RF voltage and peak intensities. Using the feature selection algorithm, the results were obtained, shown in Figure 13. This confirmed that low RF voltage did indeed generate higher peak intensities.

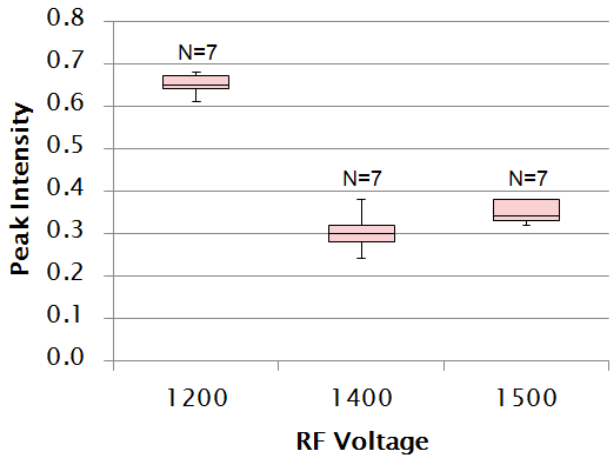


Figure 9: Deviation analysis for 50 ppm 3-carene to optimize RF voltage

Preliminary Biological Testing

Usual testing protocol dictated that, during field samples, background air samples are crucial in determining which chemicals originate from the sample, and which chemicals are present due to the surroundings. Chemicals detected in surroundings should not be considered as relevant, sample-specific biomarkers.

At the University of California, Davis, citrus trees were targeted for baseline sampling for an evaluation of device performance. Using established sampling protocols (McCartney), a 30-minute sampling of the background air was enacted, followed by an identical sampling within the canopy of the tree (see Figure 3). A shorter, less intensive sampling protocol in the past has yielded appreciable results (Aksenov).

In this particular case, however, the results were poor. The experiment was repeated on multiple trees within the same grove, as well as in two other locations (in Clovis, CA and Fairfield, CA), for a range of sampling times and a total of 43 samples (27 tree; 16 background). In general, the results, representative samples shown in Figure 14, show entirely no differentiation between background air and tree samples.

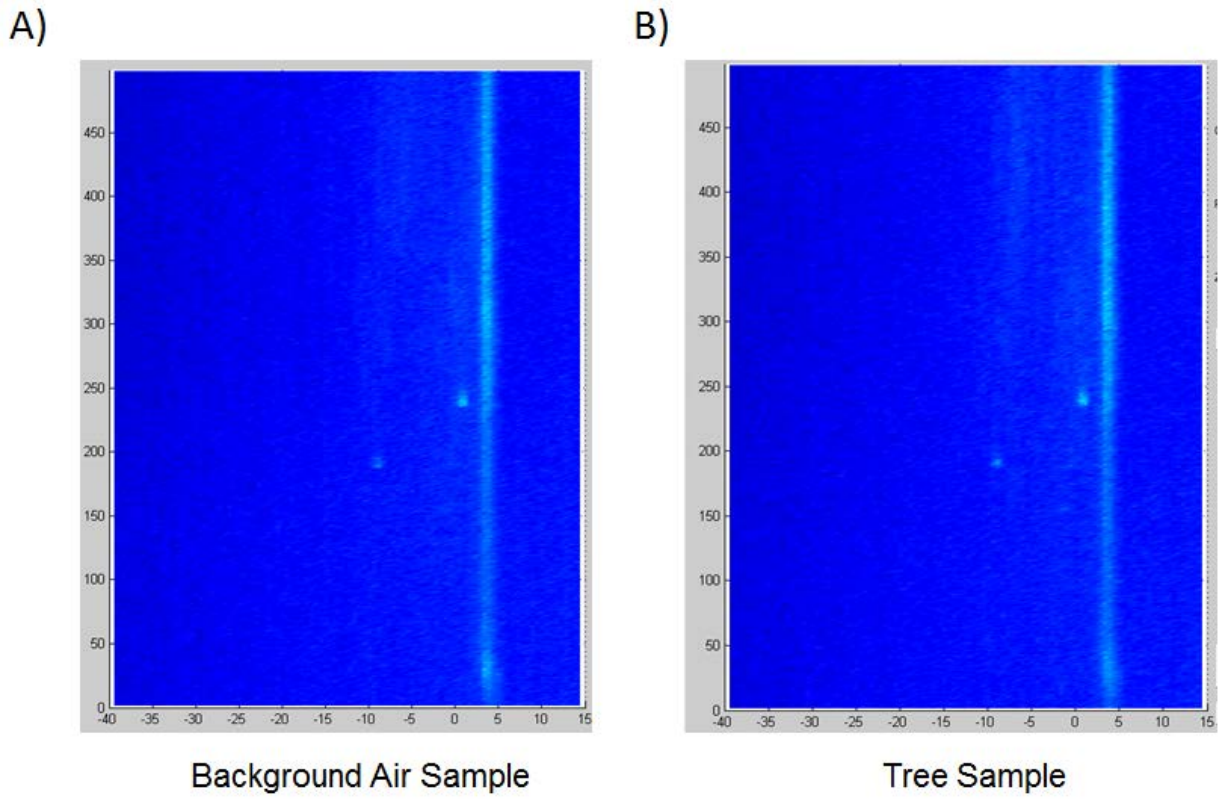


Figure 10: Representative samples of background air and trees given 30 minute sampling. A) Sample taken for 30 minutes of surrounding air. B) Tree sample taken for 30 minutes within the canopy of the tree.

The consensus on these results were that, given the range of testing, the device sensitivity was simply too poor to detect the very low concentration of VOCs emitted from the trees in natural conditions.

In an effort to determine if the nonradioactive GC/DMS was at all capable of detecting volatiles from biological plant samples, ~10 leaves near the inlet of the device were ripped (2 tears per leaf), and another 30 minute sample was taken. The damage of the leafs results in production of high abundance compounds often referred to as "green volatiles" (cut grass smell) 6-carbon alcohols/aldehydes produced due to enzymatic action upon damage of leaf's cell walls (Niinemets). The results from this are shown in Figure 15.

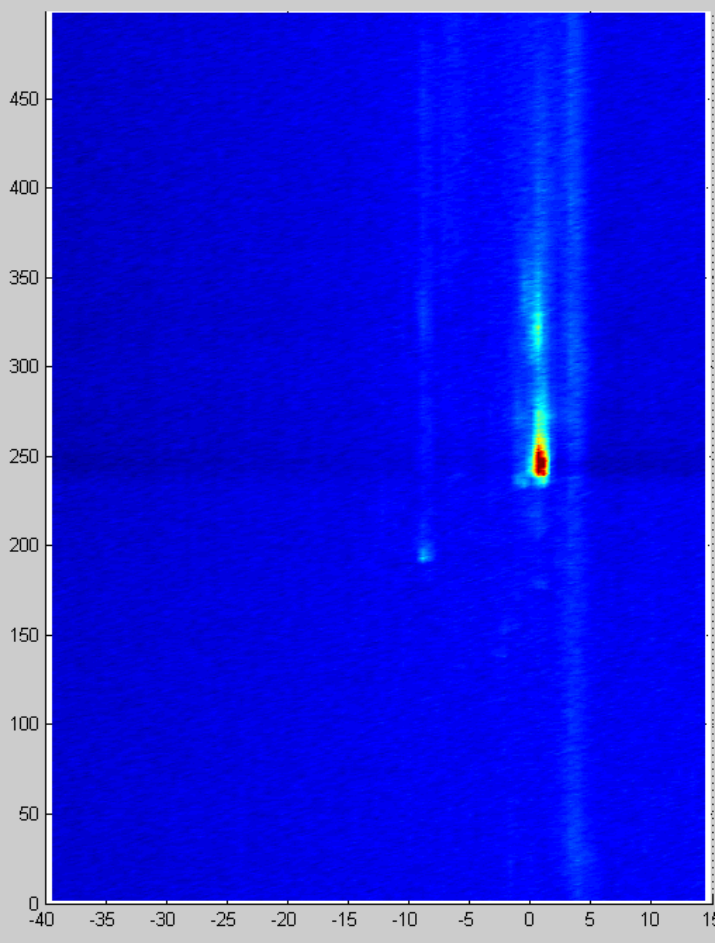


Figure 11: 30 minute Ripped leaf sample, more features detected

These results indicated that under certain circumstances, the ANI GC/DMS could detect signals from a complex biological system, but very high abundance of compounds is required. Many of the detected compounds are in parts-per-thousand and can be perceived by human olfactory system. This almost certainly the fault of the non-radioactive source.

Airflow for Enhancement of Volatile Collection

The biological testing indicated that the device was capable of detecting a measurable wounding response. Because the wounding response tends to increase green aldehyde production and drown other significant physiological biomarkers, wounding was to be avoided while working towards volatile collection enhancement. Because the current sensitivity levels are not feasible for field testing, addition of the air jet manifold uder development by the Israeli team is a critical enhancement.

Preliminary research on inducing plant motion (Nahir) and directing particulates (Gan-Mor) suggested that it was possible to stir stagnant surface volatiles in the direction of a detector to increased measured signals. Passive diffusion sampling methods within the canopy of the tree only detect those VOCs in the region of the tree where the samples are taken. For a device with poor sensitivity, those concentrations of chemicals may not be high enough for detection; thus, by using directional flow to propel volatiles to sampling inlet, detection capabilities may be higher.

For this phase of testing, a proof-of-concept experiment was implemented to determine if airflow could enhance the number and abundances of volatiles collected. In addition, a concept-only device was designed for future work on measuring airflow-induced volatiles from citrus trees.

Experimental Design for Proof-of-Concept

In order to determine if this concept was feasible and objectively assess performance increase, the experiment was carried out with plant chemicals in a controlled laboratory setting to eliminate variability associated with biological sampling.

The base experiment was set up in the following way: 9” from the inlet of the nonradioactive GC/DMS, a 1.5”x 3” piece of paper was taped down. A blank sample of air in this setup was taken before introduction of chemicals. Then, upon this piece of paper, 1 μL of methyl salicylate and 1 μL of 3-carene were simultaneously deposited. The chemicals were allowed to volatilize to equilibrium for 60 seconds. Following this 60 seconds, a 30-second sample was taken by the GC/DMS. For a control sample, this involved no alteration to the experiment layout. For an airflow sample, this involved positioning a funnel at the inlet and turning on an air compressor with an outlet flow of 200 mL/min, which was directed across the sample and towards the inlet. A diagram of both types of samples can be seen in Figure 16.

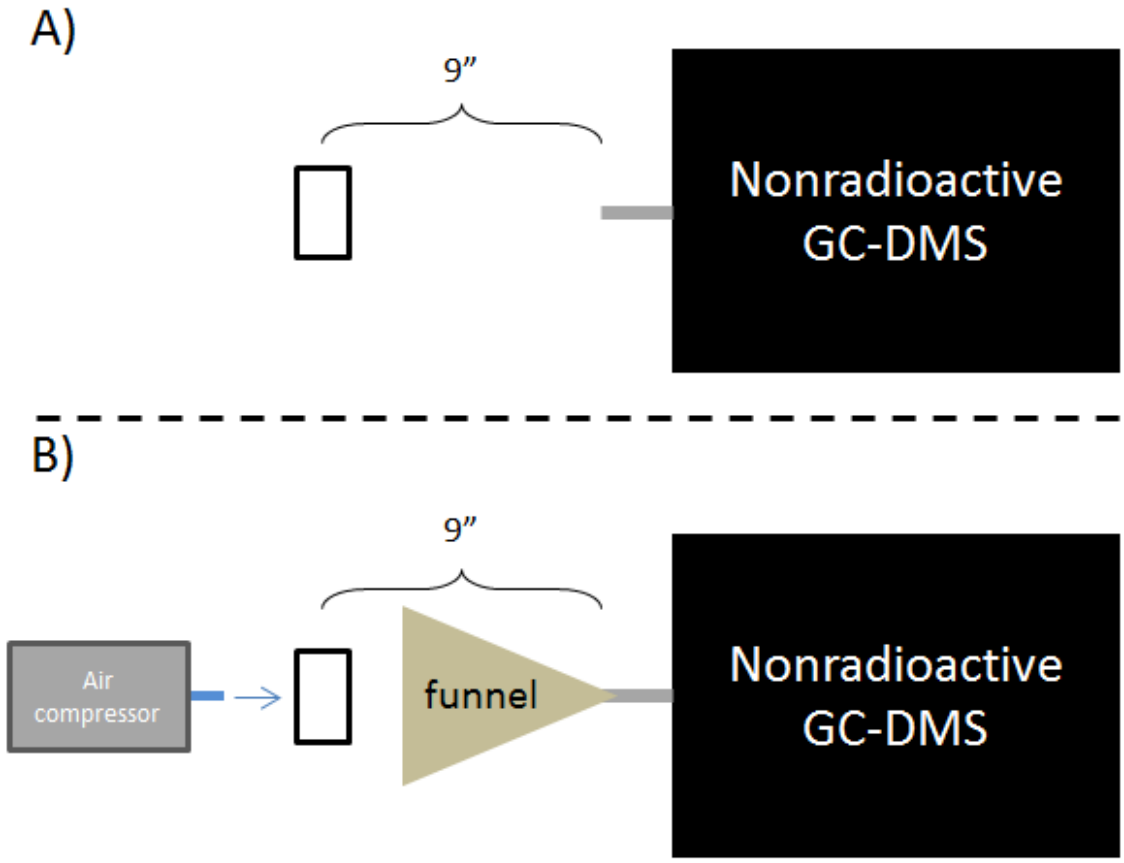


Figure 12: Airflow experiment layout. A) Control sample; B) Airflow sample using funnel and 200 mL/min flow

5.2 Results

Performing three replicates each of control samples and airflow samples, the resultant chromatograms showed a visual distinction. Averages of those samples can be seen in Figure 17.

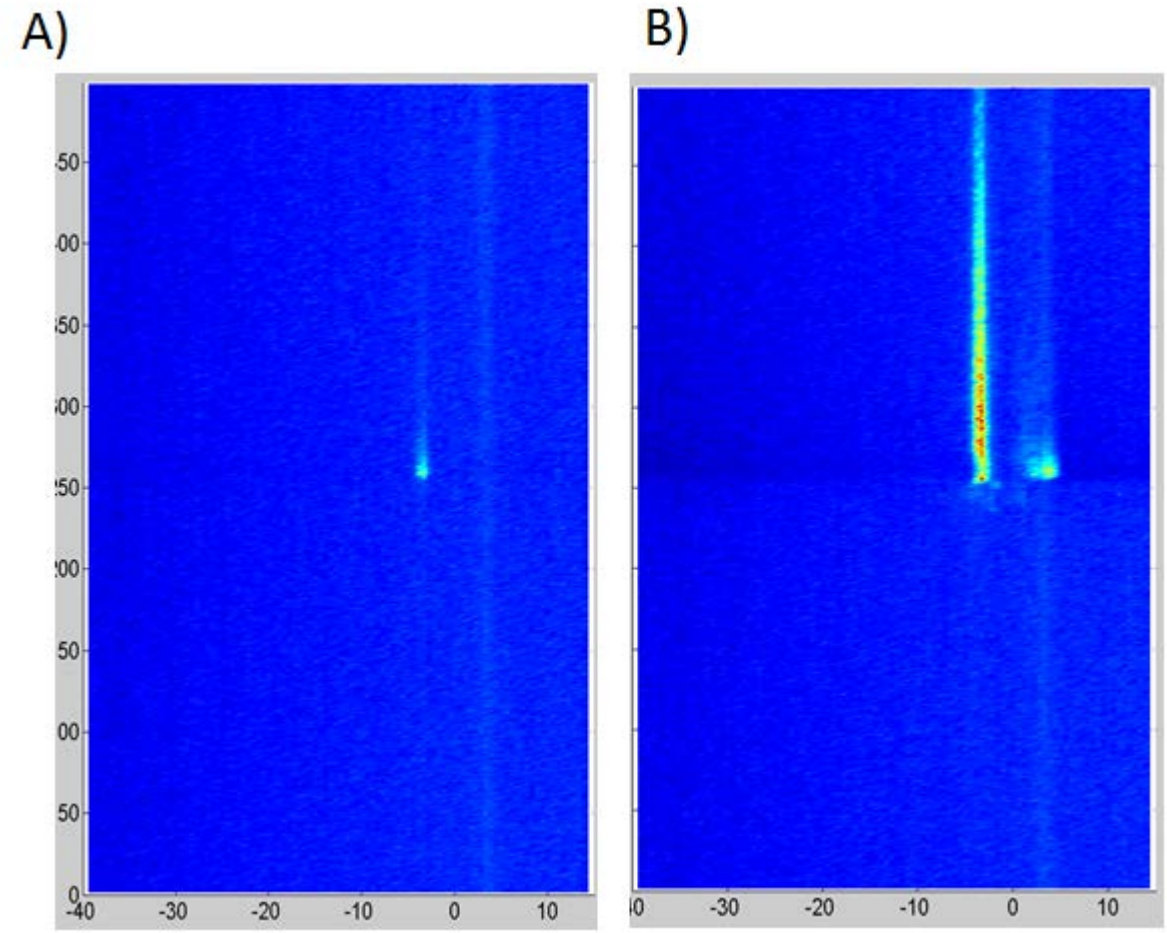


Figure 13: Results of airflow experiment. A) Control and B) airflow sample averages; N=3.

After applying the feature selection algorithm for quantification, the only feature visible in the control samples (postulated to be 3-carene based on results obtained in previous experiments) had a signal strength increase of ~3-fold at the original feature location (RT = 260 s, CV = -6 V). This can be seen in Figure 18. Excluding the tail of the suspected 3-carene feature (which the algorithm separated as a lower-intensity feature runoff), the second possible chemical feature is only seen in the airflow sample.

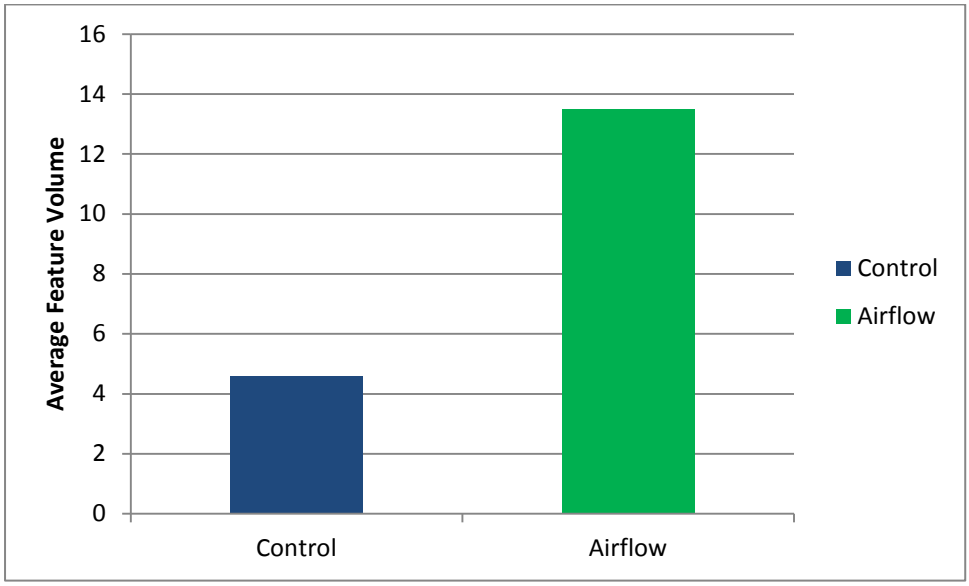


Figure 14: Quantification of airflow increase for suspected 3-carene peak

This is a very good representation of the possibilities for using airflow to aid in the collection of volatiles. As a simple proof-of-concept test, it has now been proven that airflow can increase the detected signal—even in a device with as poor of sensitivity as this particular nonradioactive GC/DMS device.

Given the success of the proof-of-concept testing with airflow, it appears to be a very promising course of action for volatile enhancement.

Draft Design

A design has been elucidated specifically to envision the assembly of the air jet manifold that will be supplied by the Israeli team. Using variable and controllable parameters such as heat and velocity, a stream of airflow will be used to stimulate the agricultural sample into outputting more volatiles, in addition to enhancing collection of existing volatiles. As this airflow system is designed for use with citrus trees, specifications are elucidated for this specific application. The adjustments for palm/RPW detection applications can be made accordingly.

Air that is ejected from an air pump will be directed through a valve in order to control the flow velocity. Following the flow control valve, the air would then be passed over a coiled heating element, which would be regulated based on the temperature of the air at the exit according to user-input parameters. After being disturbed by the air stream, volatiles that are naturally stagnant on the surface of the sample will be blown in the direction of the GC/DMS. This concept is illustrated in Figure 21.

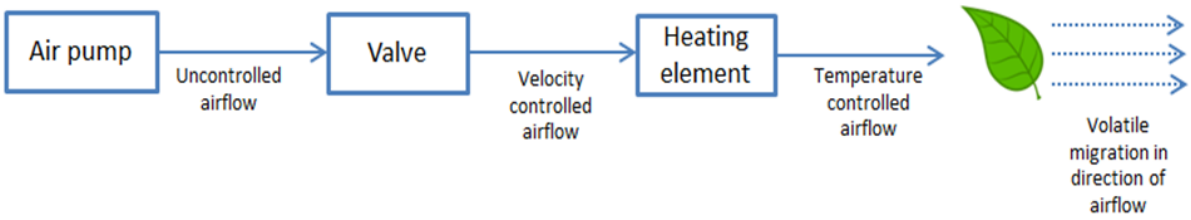


Figure 15: Air-jet system schematic with closed loop heater

Thee three primary user-controlled variables for this design are the airflow velocity, heating profile of the air, and the size of the funnel at the GC/DMS inlet. Based on research into expected basal volatile emissions, as well as documented plant response to temperature changes and wind, a concept design was created.

Funnel size: The shape of the inlet funnel is highly dependent upon tree volatile emissions. Current data of citrus trees in the California Central Valley documents emissions (Fares) between 500 and 8000 nanogram of specific compounds per gram dry leaf mass per hour (ng/g/hr). Total emissions are close to 20000 ng/g/hr. After allowing 10 leaves from 3 trees to completely dry, the leaf masses were averaged and emissions from the 3 subject trees were estimated. Total compound emissions from the target trees were estimated to be approximately 150 ng/s per leaf. Using a 900 s (15 minute) sampling time, a funnel with a volume of 2500 mL that encompassed at least 25 leaves should be able to capture enough volatiles to be detected on a 1 ppm scale. The dimensions for a 2500 mL funnel can be optimized further, but suggested dimension are shown in Figure 20, radius of 3.5” and a center length of 12”.

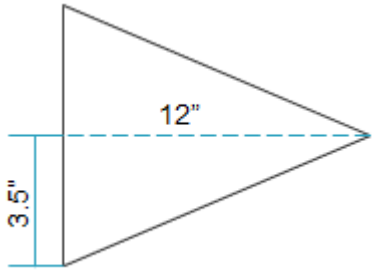


Figure 16: Suggested funnel dimensions based on California Central Valley citrus volatile emissions

Flow range: Due to variability of branch size and foliage density, the flow range should be experimentally optimized. However, in order to see a measurable response from such a small concentration of compounds, the flow should be at minimum 0.5 liters per minute (LPM). This is based on lab testing at 0.2 LPM in which a change was only

measurable for stock concentrations—much higher than those naturally present. In addition, the maximum flow should be 100 LPM (again, highly dependent on branch size and fragility), in order to prevent from inducing a wounding response.

Heat range: Research suggests that applications of heated air between 25-35°C causes plants to emit more volatiles (Joo). While this could be caused by a wounding response, the dominant compounds that increase due to temperature changes were not the usual green aldehydes indicative of damage. Exploration and optimization of applied temperatures would be ideal, but a good starting point would be slightly higher than ambient temperatures.

Data Loggers for Humidity and Temperature Monitoring

GC/DMS devices specifically can be very susceptible to changes in humidity and temperature. Hydronium ions in the gas phase that serve as the charge reservoir by transfer charge to neutrals. The size of these clusters and gas-phase charge transfer equilibria are affected by multitude of parameters, such as humidity and ambient temperature. Shifts in these equilibria, in turn would affect target analyte ion structures and abundances. In addition, chemical reactions such as dissociation and charge change may take place as a result of certain instrumental conditions. For these reasons, it would be beneficial to accompany the future airflow design with some type of data logger. In the past, data loggers that use digital temperature and humidity sensor probes (Part AM2315, Aosong Electronics CO, Ltd., Guangzhou China) were used in conjunction with microcontrollers that recorded data to SD cards [McCartney]. Using a similar technology would allow for closer measurement of the humidity and temperature levels the plants (and subsequently the GC/DMS) experienced.

For previous devices preassembled by another lab member, original scripts were been constructed to read and compile the data into a single Excel file, in addition to generating plots based on days (shown in Figures 21 and 22). Plots for temperature and humidity data based on location of the probes in or out of enclosure would now be based on probe location in the system's funnel or in surrounding environment.

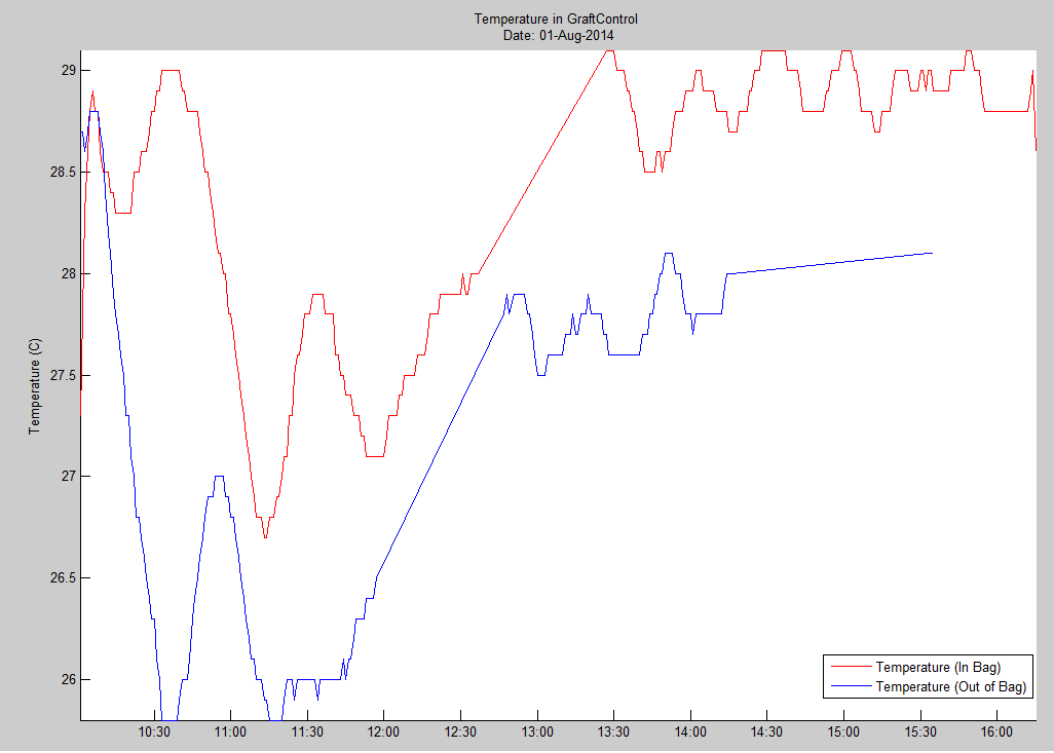


Figure 17: Temperature output of data logger

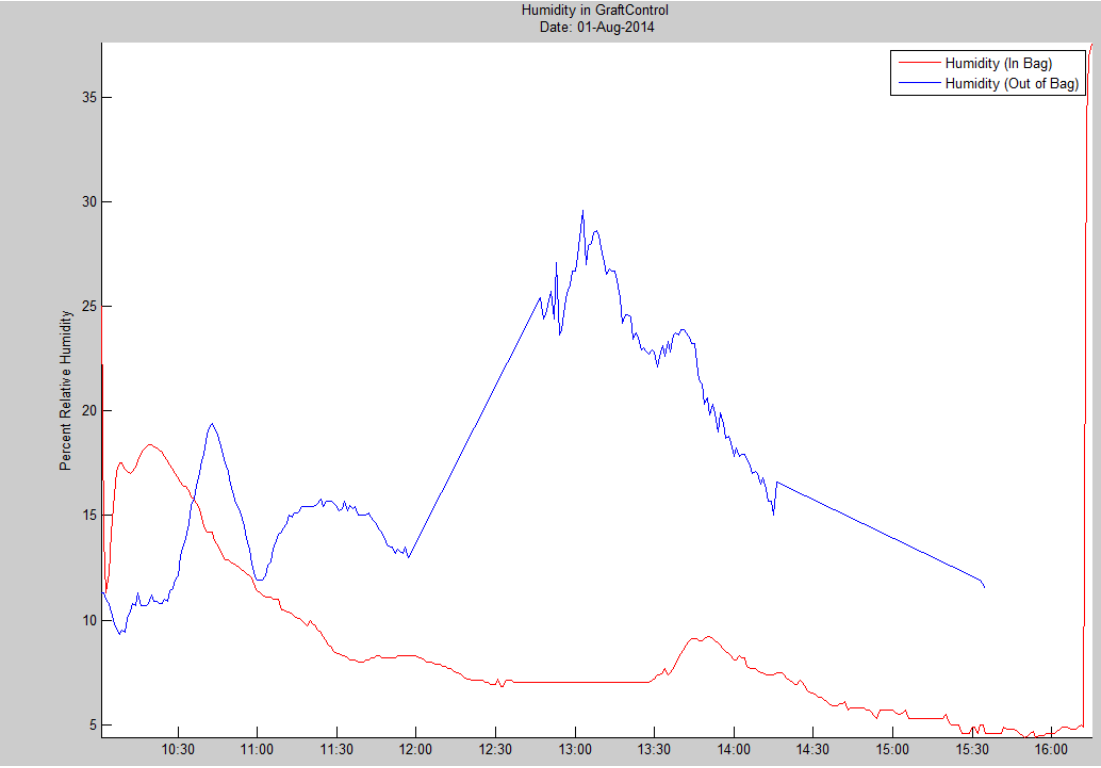


Figure 18: Humidity output of data logger

Custom Enclosure for Isolate and Study Date Palm Volatiles

Management of any pest requires accurate monitoring of its population, forecasting its dispersal and evaluating the success of eradication efforts. As the best management is prevention, preclusion of further spread of invasive pests requires tools for monitoring, especially at ports of entry and at new infestation foci. Early detection of the infestation by the red palm weevil (RPW), *Rhynchophorus ferrugineus* Olivier, 1790, is particularly challenging as the pest develops inside the palm, well hidden from the human eye. Various methods and approaches have been evaluated over the years for early detection. The most obvious approach for infestation detection is visual examination of the tree. But visual detection of RPW activity depends mainly on infestation stage, the site of infestation, palm height and species. For example, crown infestation by RPW is easiest to detect as the palm crown loses its symmetry and inner fronds show chewing symptoms. This situation is common with Canary Island date palm (*Phoenix canariensis*) and coconut (*Cocos nucifera* L.), but rather rare in date palms (*Phoenix dactylifera* L.). In the latter, the infestation occurs mostly in the lower part of the stem; if offshoots are present, the symptoms appear either on the offshoots themselves or as tunnels and cavities within the stem, often in places of previously removed offshoots. The palm may appear healthy, without any visible symptoms, until the damage to the trunk tissues overpowers the tree and it collapses, exposing large cavities. Hidden activity of these pests makes visual detection of the infestation problematic and inaccurate. On the other hand, pest development within the palm tissue is expected to be associated with both pest-specific traces (such as distinct odors or sounds) and specific physiological changes in the palm that are invisible to the naked eye, but can be identified by other means, such as acoustic thermal or sniffing dogs (Mankin, 2011; Soroker et al., 2013; Suma et al., 2014). Methods developed so far rarely reached 90-80% precision and each possess its technical or physiological limitations (Soroker et al., 2016). The fact that chemical cues produced by plants in response to herbivore attack is well known phenomena (Dudareva et al., 2013) along with the reported ability of sniffing dogs to detect infected palms suggests that electronic nose or tongue can be a feasible detection approach. Although palm volatiles were previously analyzed in palm tissues (Rochat et al., 2000; Vacas et al., 2014) no information regarding volatile profile of date palms and any growing palms infested or even healthy is yet unavailable. This work aimed at identification of specific volatiles emitted by date palm infested by the red palm weevils.

After a failure of an initial collection system, we have made changes and the current system is presented in Figure 23.

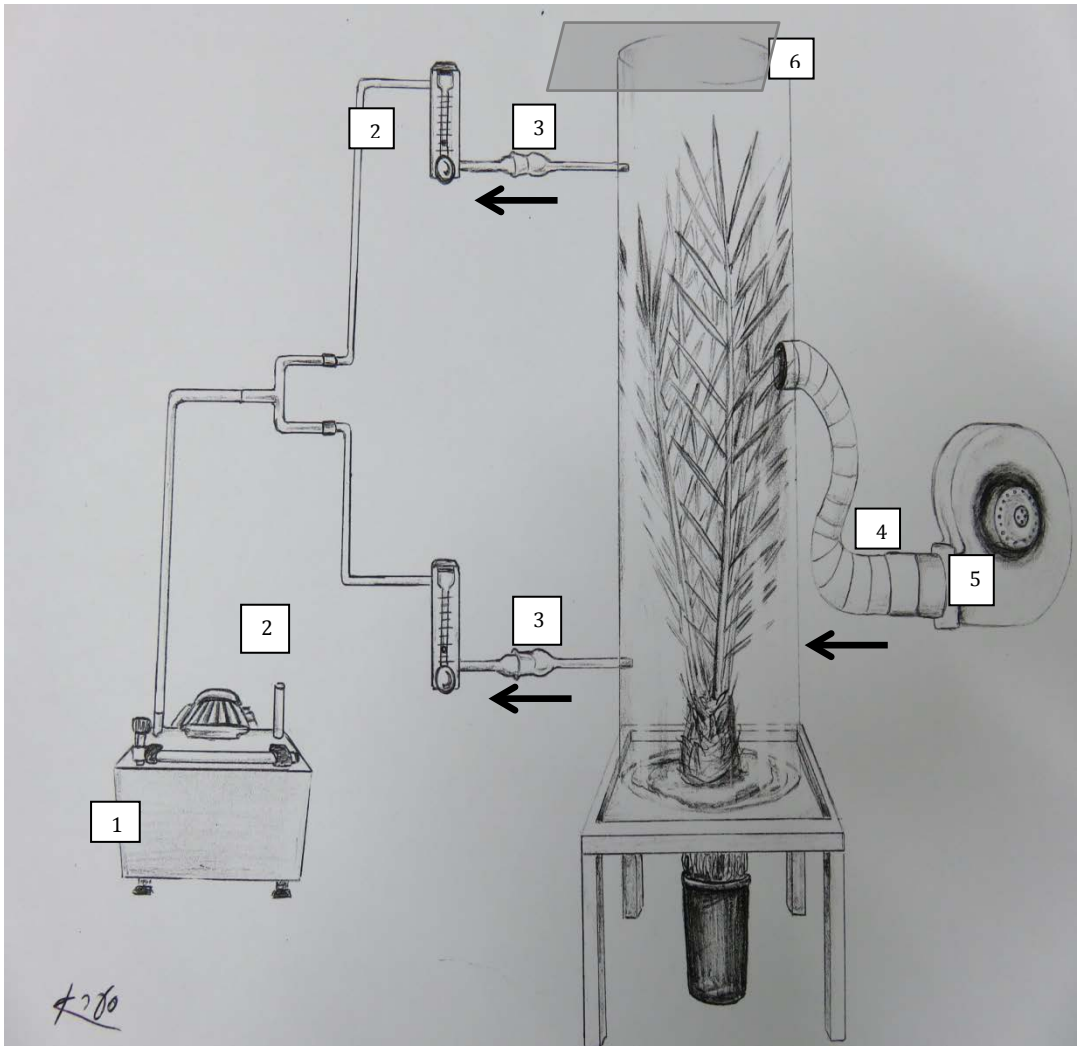


Figure 23: The head space collection set up: 1. Vacuum pump (GmbH Greiffenberger Antriebstechnik, Marktredwitz, Germany.; 2. Flow meter set at 1 liter/ min. 3. SuperQ column; 4. Charcoal filter 5. Air blower (Ebm-Papst, Mulfingen, Germany) 6. Cover glass. The height of the system is 190 cm, the cylinder size (50 cm diameter and 150 cm height). Arrows indicate direction of air flow.

Methodology

We collected volatiles from growing seed born young date palms *Phoenix dactilifera* as these were the only untreated palms available. After several adaptations of the volatile collecting system the scheme of the final system is presented on Figure 23.

Volatile collection setup

Following acclimatization of the palms (150-190cm height, 17-20cm diameter) for a day or two in the collection room head space collection was conducted for 24 hours, twice (day after day). After two air collection from uninfested palms the palms were artificially infested with

five ,*R. ferrugineus larvae* 20-40 mg each. Larvae were introduced one by one into pre-drilled hole in the leaf bases.

The air sampling were repeated at additional times: 5-6 day, 21-22 day, 33-34 day, 54-55 day or till palms died. The environmental conditions were kept stable: constant light and about 27 ºC.

B. Volatile collection

Volatiles were collected on self-made glass columns loaded with 300mg Super Q 80/100) Alltech Associates, Deerfield, IL. This type of absorbent was selected due to its ability to trap a variety of plant volatiles (Alltech, 2003). eg. terpens from monocots such as maize (Schnee et al., 2006) or dicots such as soya (Sun et al., 2014).

Two columns were used at each time period set at two heights about 30 cm and 100 cm above palm ground. This set up aimed to collect both leaf and trunk derived volatiles. The volatiles were extracted with 1.5 ml DCM (for spectroscopy, Merck). Samples were concentrated to 100 microliters for injection to GC-FID. One microliter of sample was used for analysis. Mix of hydrocarbons C9-C33 was regularly used as external standards for calculations of Kovats Index.

C. GC and GC-MS analysis

GCFID (Thermo Scientific), column: Restek RXi-5MS - 30 meters, 0.25 mm ID.
Detector T=270 ºC; Inlet T=260 ºC; Oven program: 45ºC (2min) to 100 (5 ºC/min) to 270 (10 ºC/min, 5min)

GC-MS (Agilent Technologies(5890B) USA) column:Agilent HP-5MS 5% phenyl methyl silox 30mX0.25mmX0.25µm.

D. Peak identification and statistical analysis:

Peak integration was done using Chromeleon 7.2 CDS. The peaks of interest were identified by GC-MS with the help of MassHunter software, NIST library and Kovats index.

Results and Discussion

We have conducted regular volatile collection from five young date palms. In addition we also collected volatiles from two date palms and two *Washingtonian sp*. previously infected with RPW larvae, as the other experimental palms but were grown in greenhouse for about 30d till

volatile collection. Overall the amount of volatiles, produced by date palms over 24 hours collection, are quite small (about 1µg total per collection on average). Figure 24 shows chromatogram of volatiles collected from the uninfected palm relative to volatiles collected from empty chamber. On average 100 peaks per chromatogram was observed. The fact that the peaks are spread all along the chromatogram indicates that the volatile profile is very heterogeneous in term of volatility. Not much difference was observed between volatiles collected from upper and lower part of the chamber.

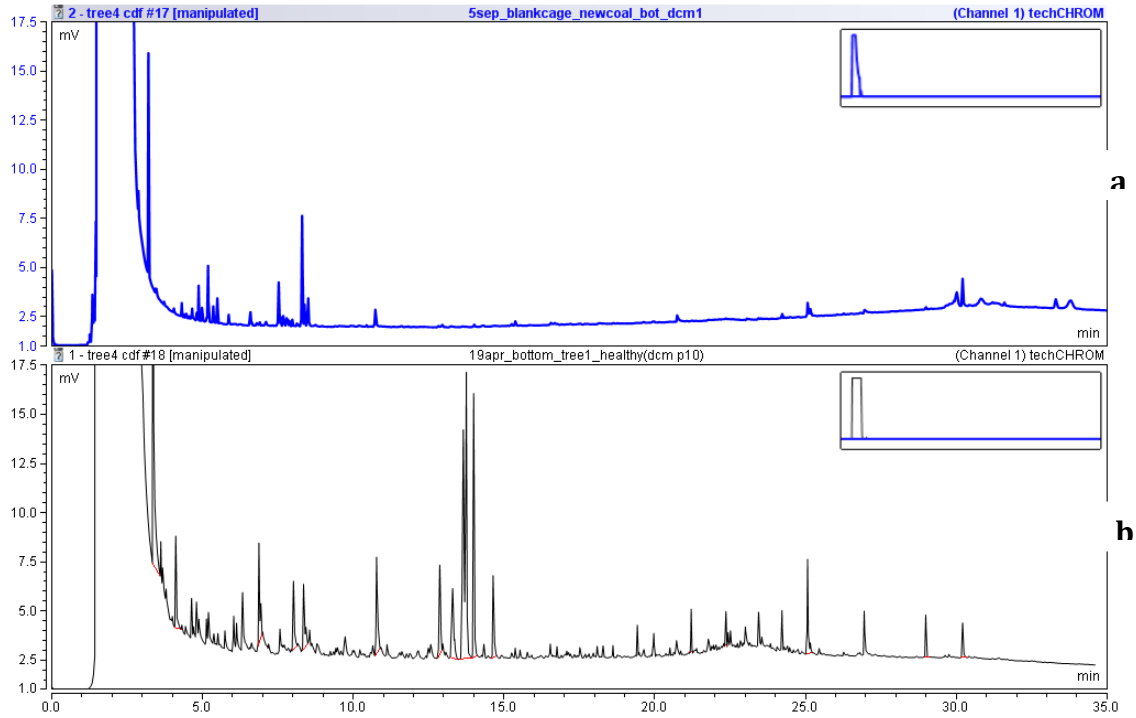


Figure 24. The GC profile of empty collection chamber and uninfested date palm 1 (below).

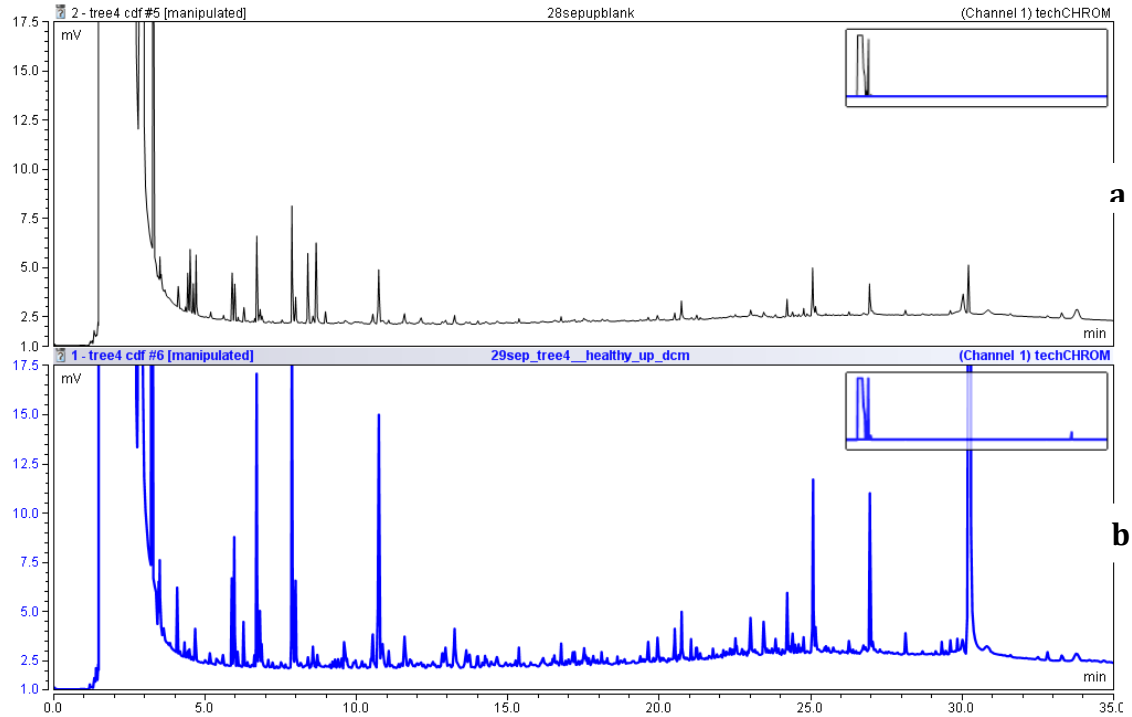


Figure 25. The GC profile of empty collection chamber and uninfested date palm 2 (below).

As can be seen from comparison of figures 24 and 25, the volatile profile of uninfested palms was somewhat variable. This is not surprising as palms were seed born and not offshoots of specific variety.

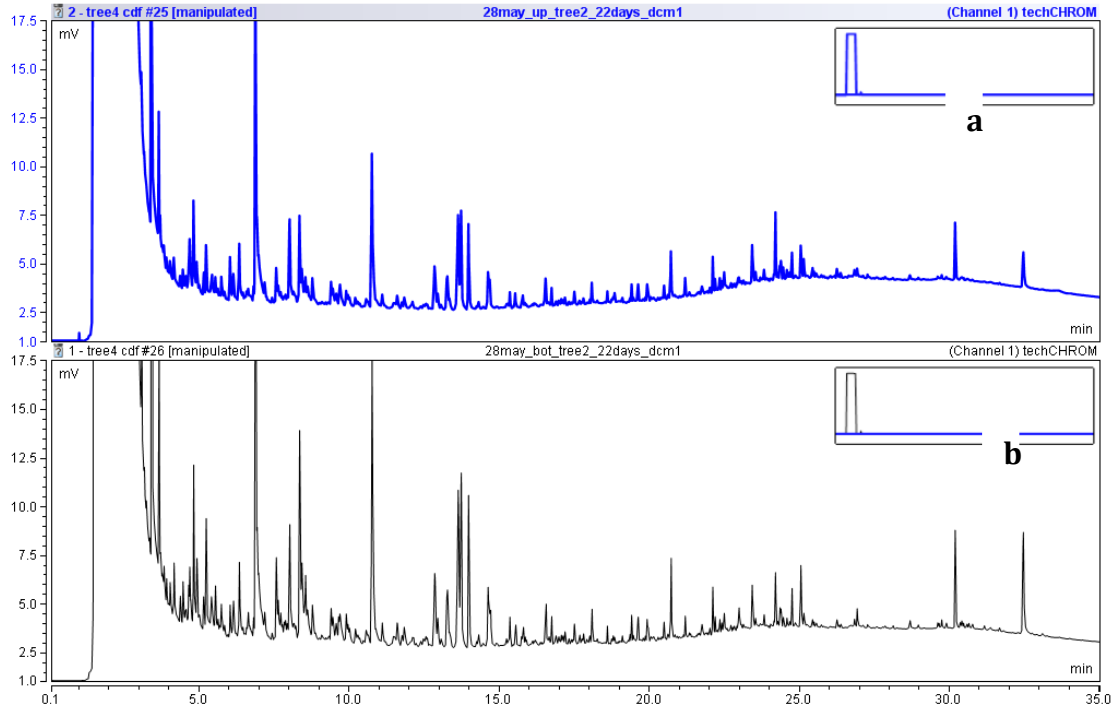
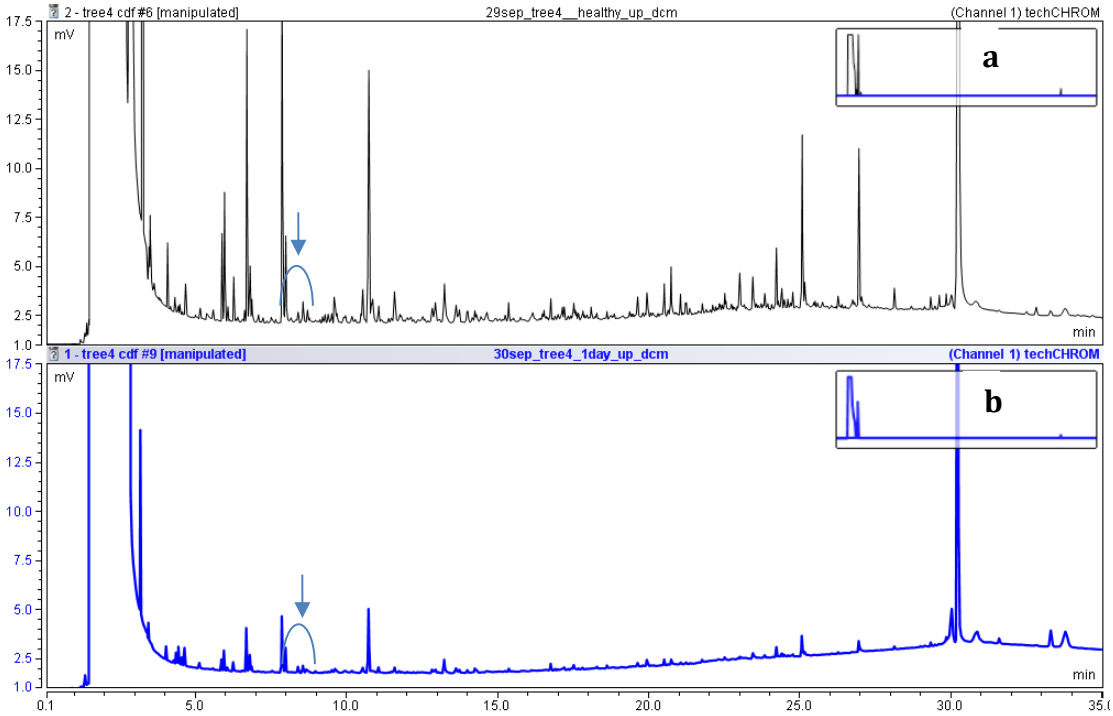


Fig 26. Volatile profile of the same date palm #2 collected 22 days post infection from upper trap (a) and lower trap (b).

As can be expected following infection with red palm weevils the volatile profile palms changed. The example for such time dependent change is shown on Figure 27. The fact that the amount of volatiles decreased in tree #4 following infestation can be probably explained by decrease in leave number following infestation (as these were cut for infestation purposes).

GCMS analysis and kovats indices enabled us to identify two compounds that are dramatically prominent in the weevil infested palms (Table 5): these are hydroperoxide 1-ethylbutyl and hydroperoxide 1-methylpentyl. It is important to indicate that these compounds appeared about a week after infestation before any symptoms on palms are visible. There amount increases towards three weeks post infestation, but subsequently decreases as palm deteriorates.

The source of this compounds is yet unknown. These chemicals were reported as part of plant volatiles in several plant species eg *Moringa peregrina* leaves (Al-Owaisi et al., 2014) flower fragrance in orchid *Cypripedium flavum* (Orchidaceae) (Zheng et al., 2011), Durian fruit fragarance *Durio ziberthinus* (Jiang et al., 1998). However, these chemicals were not previously reported from palms.



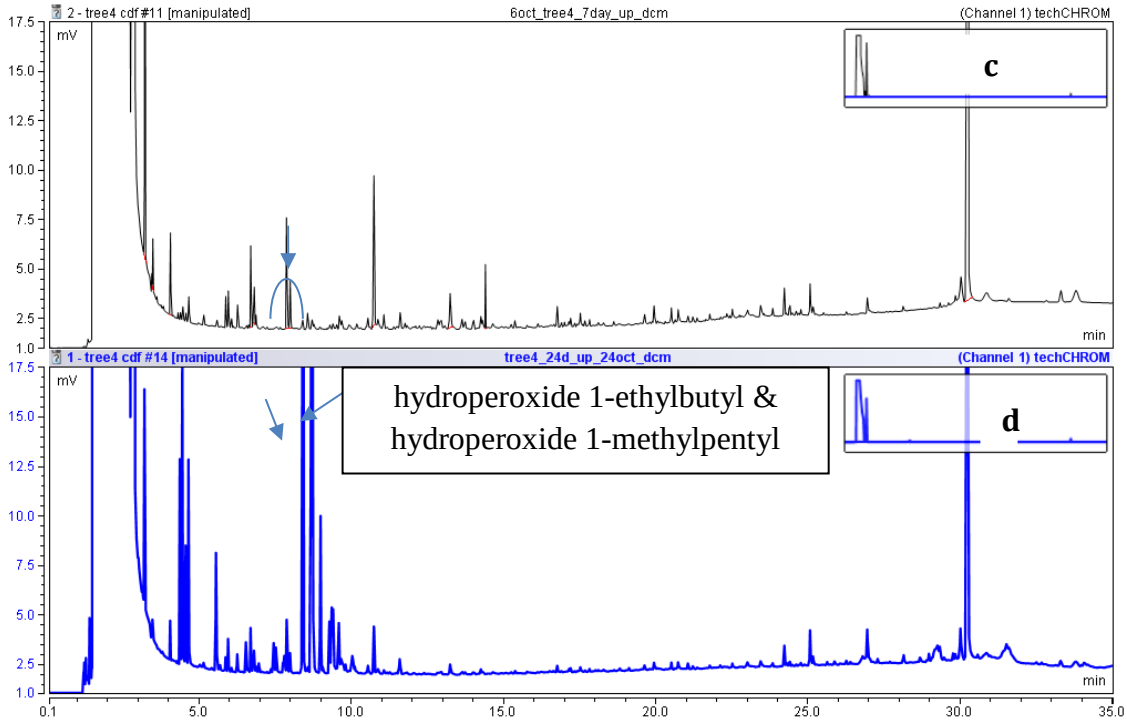


Figure 27. Comparison of volatile profiles collected from palm 4 at different time periods: a. Prior to infestation; b. One day after infestation; c. Seven days after infestation and d. 24 days after infestation. Arrows indicate peaks of interest.

Conclusions

One year of study enabled us to develop a system for head space collection from a growing date palm and identify two volatiles that potentially indicate RPW infection in palms. However more study is needed in order to proceed with volatile identification and further confirm identification of volatiles tentatively identified in this study.

Table 5: Compounds tentatively identified mostly in infected palms

IUPAC name[#]	**RI**	**% In healthy date palms** Mean ± SD n=3	**% In infected date palms 21-34 DPI** Mean ± SD **n=7**	**% In infected Washingtonia 30 DPI** Mean ± SD **n=2**	**Identified in infested palms at quantity > 1%**
hydroperoxide 1-ethylbutyl	948	0.31±0.29	6.22±7.83	4.66±4.51	Date 1,2, 3, 4, 10*,11* *Washingtonia* #1 & #2
hydroperoxide 1-methylpentyl	957	0.18±0.32	6.74±10.25	4.59±6.49	Date 3,4, 10* *Washingtonia* #1

RI= retention index; DPI- day post infection.
*infested palms from greenhouse

#Tentative with spectra and high probability matches >80% according to NICT mass spectral library.

References

Aksenov, Alexander A., Alberto Pasamontes, Daniel J. Peirano, Weixiang Zhao, Abhaya M. Dandekar, Oliver Fiehn, Reza Ehsani, and Cristina E. Davis. "Detection of Huanglongbing Disease Using Differential Mobility Spectrometry." *Analytical Chemistry Anal. Chem.* 86.5 (2014): 2481-488. Web.

Alltech (2003). Gas Cromatography. Available at http://www.axialscientific.com/files/alltech_gc_packed.pdf.

Al-Owaisi, M., Al-Hadiwi, N., and Khan, S.A. (2014). GC-MS analysis, determination of total phenolics, flavonoid content and free radical scavenging activities of various crude extracts of Moringa peregrina (Forssk.) Fiori leaves. Asian Pac. J. Trop. Biomed. 4, 964–970.

Anderson, Andrew G., Kenneth A. Markoski, Quan Shi, Stephen L. Coy, Evgeny V. Krylov, and Erkinjon G. Nazarov. "DMS-IMS2, GC-DMS, DMS-MS: DMS Hybrid Devices Combining Orthogonal Principles of Separation for Challenging Applications." *Chemical, Biological, Radiological, Nuclear, and Explosives (CBRNE) Sensing IX* (2008): n. pag. Web.

Anderson, Andrew G., Kenneth A. Markoski, Quan Shi, Stephen L. Coy, Evgeny V. Krylov, and Erkinjon G. Nazarov. "DMS-IMS2, GC-DMS, DMS-MS: DMS Hybrid Devices Combining Orthogonal Principles of Separation for Challenging Applications." *Chemical, Biological, Radiological, Nuclear, and Explosives (CBRNE) Sensing IX* (2008): n. pag. Web.

Baldwin, I. T. "Volatile Signaling in Plant-Plant Interactions: "Talking Trees" in the Genomics Era." *Science* 311.5762 (2006): 812-15. Web.

Barnett, David A., Roger Guevremont, and Randy W. Purves. "Determination of Parts-per-Trillion Levels of Chlorate, Bromate, and Iodate by Electrospray Ionization/High-Field Asymmetric Waveform Ion Mobility Spectrometry/Mass Spectrometry." *Applied Spectroscopy Appl Spectrosc* 53.11 (1999): 1367-374. Web.

Bohon, Robert L., and W. F. Claussen. "The Solubility of Aromatic Hydrocarbons in Water 1." J. Am. Chem. Soc. Journal of the American Chemical Society 73.4 (1951): 1571-578. Web.

Buttery, Ron G., Louisa Ling, and Dante G. Guadagni. "Food Volatiles: Volatilities of Aldehydes, Ketones, and Esters in Dilute Water Solution." J. Agric. Food Chem. Journal of Agricultural and Food Chemistry 17.2 (1969): 385-89. Web.

Dudareva, N., Klempien, A., Muhlemann, J.K., and Kaplan, I. (2013). Biosynthesis, function and metabolic engineering of plant volatile organic compounds. New Phytol. 198, 16–32.

Eiceman, Gary Alan., Zeev Karpas, and H. H. Hill. *Ion Mobility Spectrometry*. Boca Raton, FL: CRC, 2012. Print.

Eiceman, G. A., E. G. Nazarov, R. A. Miller, E. V. Krylov, and A. M. Zapata. "Micro-machined Planar Field Asymmetric Ion Mobility Spectrometer as a Gas Chromatographic Detector." *The Analyst Analyst* 127.4 (2002): 466-71. Web.

Eiceman, G. A, B. Tadjikov, E. Krylov, E.g Nazarov, R.a Miller, J. Westbrook, and P. Funk. "Miniature Radio-frequency Mobility Analyzer as a Gas Chromatographic Detector for Oxygen-containing Volatile Organic Compounds, Pheromones and Other Insect Attractants." *Journal of Chromatography A* 917.1-2 (2001): 205-17. Web.

Fares, Silvano, Drew R. Gentner, Jeong-Hoo Park, Elena Ormeno, John Karlik, and Allen H. Goldstein. "Biogenic Emissions from Citrus Species in California." Atmospheric Environment 45.27 (2011): 4557-568. Web.

Gan-Mor, S., Y. Riven and A. Grinstein. “Improving pesticide deposits in open tomato fields by directed air-streams.” (1998) EurAgEng Paper No. 98-A-009, Oslo, Norway.

Handy, Russell, David A. Barnett, Randy W. Purves, Gary Horlick, and Roger Guevremont. "Determination of Nanomolar Levels of Perchlorate in Water by ESI-FAIMS-MS." *Journal of Analytical Atomic Spectrometry J. Anal. At. Spectrom.* 15.8 (2000): 907-11. Web.

Harper, Martin. "Sorbent Trapping of Volatile Organic Compounds from Air." *Journal of Chromatography A* 885.1-2 (2000): 129-51. Web.

Jiang, J., Choo, S.Y., Omar, N., and Ahamad, N. (1998). GC-MS analysis of volatile compounds in durian (Durio zibethinus Murr.). In Developments in Food Science, C.-T.H. E.T. Contis C.J. Mussinan, T.H. Parliment, F. Shahidi and A.M. Spanier, ed. (Elsevier), pp. 345–352.

Joó, É., J. Dewulf, C. Amelynck, N. Schoon, O. Pokorska, M. Šimpraga, K. Steppe, Marc Aubinet, and H. Van Langenhove. "Constitutive versus Heat and Biotic Stress Induced BVOC Emissions in Pseudotsuga Menziesii." Atmospheric Environment 45.22 (2011): 3655-662.

Kessler, A. "Defensive Function of Herbivore-Induced Plant Volatile Emissions in Nature." *Science* 291.5511 (2001): 2141-144. Web.

Kolakowski, Beata M., and Zoltán Mester. "Review of Applications of High-field Asymmetric Waveform Ion Mobility Spectrometry (FAIMS) and Differential Mobility Spectrometry (DMS)." *The Analyst Analyst* 132.9 (2007): 842. Web.

Louis, Robert H. St., Herbert H. Hill, and Gary Alan Eiceman. "Ion Mobility Spectrometry in Analytical Chemistry." *Critical Reviews in Analytical Chemistry* 21.5 (1990): 321-55. Web.

Mankin, R.W. (2011). Recent Developments in the use of Acoustic Sensors and Signal Processing Tools to Target Early Infestations of Red Palm Weevil in Agricultural Environments. Fla. Entomol. 94, 761–765.

McCartney, Mitchell M., Sierra L. Spitulski, Alberto Pasamontes, Daniel J. Peirano, Michael J. Schirle, Raquel Cumeras, Jason D. Simmons, Jeffrey L. Ware, Joshua F. Brown, Alexandria J.y. Poh, Seth C. Dike, Elizabeth K. Foster, Kristine E. Godfrey, and Cristina E. Davis. "Coupling a Branch Enclosure with Differential Mobility Spectrometry to Isolate and Measure Plant Volatiles in Contained Greenhouse Settings." *Talanta* 146 (2016): 148-54. Web.

Michels, Antje, Sven Tombrink, Wolfgang Vautz, Manuela Miclea, and Joachim Franzke. "Spectroscopic Characterization of a Microplasma Used as Ionization Source for Ion Mobility Spectrometry." *Spectrochimica Acta Part B: Atomic Spectroscopy* 62.11 (2007): 1208-215. Web.

Nahir, D., S. Gan-Mor, Irena Rylski, and H. Frankel. "Pollination of Tomato Flowers by a Pulsating Air Jet." *Transactions of the ASAE* 27.3 (1984): 894-96. Web.

Niinemets, Ülo, and R. K. Monson. Biology, Controls and Models of Tree Volatile Organic Compound Emissions. Dordrecht: Springer, 2013. Print.

Peirano, Daniel, Alexander Aksenov, Alberto Pasamontes, and Cristina Davis. "Approaches for Establishing Methodologies in Metabolomic Studies for Clinical Diagnostics." *Medical Applications of Artificial Intelligence* (2013): 279-306. Web.

Peirano, Daniel, et al. “Supervised Semi-Automated Data Analysis Software for Gas Chromatography / Differential Mobility Spectrometry (GC/DMS) Metabolomics Applications.” *International Journal for Ion Mobility Spectrometry* (2016): 19(2): 155-166. Web.

Riddick, John A., William B. Bunger, and Arnold Weissberger. Organic Solvents; Physical Properties and Methods of Purification. New York: Wiley-Interscience, 1970. Print.

Rochat, D., Meillour, P.N.-L., Esteban-Duran, J.R., Malosse, C., Perthuis, B., Morin, J.-P., and Descoins, C. (2000). Identification of Pheromone Synergists in American Palm Weevil, Rhynchophorus Palmarum, and Attraction of Related Dynamis Borassi. J. Chem. Ecol. 26, 155–187. Web.

Rose, A. "Vision: Human and Electronic." *Applied Solid State Physics* (1970): 79-160. Web.
Rowland, FW. “What Is Gas-Chromatography,” Hewlett-Packard J, vol. 34, no. 1, 1983, pp. 32-33. Web.

Schilmiller, Anthony L., and Gregg A. Howe. "Systemic Signaling in the Wound Response." *Current Opinion in Plant Biology* 8.4 (2005): 369-77. Web.

Schnee, C., Köllner, T.G., Held, M., Turlings, T.C.J., Gershenzon, J., and Degenhardt, J. (2006). The products of a single maize sesquiterpene synthase form a volatile defense signal that attracts natural enemies of maize herbivores. Proc. Natl. Acad. Sci. U. S. A. 103, 1129–1134. Web.

Soroker, V., Suma, P., Pergola, A.., Llopis, V.., Vacas, S., Cohen, Y., Alchanatis, V., Milonas, P., Golomb, O., Goldshtein, E., et al. (2016). Surveillance techniques and detection methods for Rhynchophorus ferrugineus and Paysandisia archon. Web.

Soroker V Colazza Eds Handb. Rhynchophorus Ferrugineus Paysandisia Archon– Biol. Manag. Willes. Suma, P., Pergola, A.L., Longo, S., and Soroker, V. (2014). The use of sniffing dogs for the detection of Rhynchophorus ferrugineus. Phytoparasitica 42, 269–274. Web.

Soroker, V., Suma, P., Pergola, A. la, Cohen, Y., Alchanatis, V., Golomb, O., Goldshtein, E., Hetzroni, A., Galazan, L., Kontodimas, D., et al. (2013). Early detection and monitoring of red palm weevil: approaches and challenges. In Colloque Méditerranéen Sur Les Ravageurs Des Palmiers, Nice, France, 16-18 Janvier 2013, (Association Française de Protection des Plantes (AFPP)). Print.

Sumner, Lloyd W., Pedro Mendes, and Richard A. Dixon. "Plant Metabolomics: Large-scale Phytochemistry in the Functional Genomics Era." *Phytochemistry* 62.6 (2003): 817-36. Web.

Sun, X., Liu, Z., Zhang, A., Dong, H.-B., Zeng, F.-F., Pan, X.-Y., Wang, Y., and Wang, M.-Q. (2014). Electrophysiological responses of the rice leaffolder, Cnaphalocrocis medinalis, to rice plant volatiles. J. Insect Sci. 14, 70. Web.

Synge, Richard LM. "Applications of Partition Chromatography." Nobel Lecture. 12 Dec. 1952. *Nobel Lectures in Chemistry 1943-1962*. River Edge, NJ: World Scientific, 1999. 374-87. Print.

Tholl, Dorothea, Wilhelm Boland, Armin Hansel, Francesco Loreto, Ursula S.r. Röse, and Jörg-Peter Schnitzler. "Practical Approaches to Plant Volatile Analysis." *The Plant Journal* 45.4 (2006): 540-60. Web.

Tikhonski, Alexei, and Leif Thuesen. Nonradioactive Ionization Source Driver. PEN, Inc, Deerfield Beach, FL (US), assignee. Patent US9111738B2. 18 Aug. 2015. Print.

Vacas, S., Abad-Payá, M., Primo, J., and Navarro-Llopis, V. (2014). Identification of pheromone synergists for Rhynchophorus ferrugineus trapping systems from Phoenix canariensis palm volatiles. J. Agric. Food Chem. 62, 6053–6064. Web.

Zheng, G., Li, P., Pemberton, R., and Luo, Y. (2011). Mixed bumblebee and blowfly pollination of Cypripedium flavum (Orchidaceae) in Sichuan, China. Ecol. Res. 26, 453–459. Web.

Zini, Cláudia A., Fabio Augusto, Eva Christensen, Elina B. Caramão, and Janusz Pawliszyn. "SPME Applied to the Study of Volatile Organic Compounds Emitted by Three Species of Eucalyptus in Situ." *J. Agric. Food Chem. Journal of Agricultural and Food Chemistry* 50.25 (2002): 7199-205. Web.